

# *Enhancement of glucosinolate and isothiocyanate profiles in brassicaceae crops: addressing challenges in breeding for cultivation, storage, and consumer-related traits*

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**Enhancement Of Glucosinolate & Isothiocyanate Profiles In Brassicaceae Crops:  
Addressing Challenges In Breeding For Cultivation, Storage, and Consumer Related  
Traits**

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## ABSTRACT

Glucosinolates (GSLs) and isothiocyanates (ITCs) produced by Brassicaceae plants are popular targets for analysis due to the health benefits associated with them. Breeders aim to increase the concentrations in commercial varieties, however there are few examples of this. The most well known is *Beneforté* broccoli, which has increased glucoraphanin/sulforaphane concentrations compared to conventional varieties. It was developed through traditional breeding methods with considerations for processing, consumption and health made throughout this process. Many studies presented in the literature do not take a holistic approach, and key points about breeding, cultivation methods, postharvest storage, sensory attributes and consumer preferences are not properly taken into account. In this review, we draw together data for multiple species and address how such factors can influence GSL profiles. We encourage researchers and institutions to engage with industry and consumers to produce research that can be utilised in the improvement of Brassicaceae crops.

Keywords: Brassica, Phytochemicals, Plant breeding, Nutrition, Processing, Chemoprotection, Glucoraphanin, Indoles, Broccoli, Cabbage, Mustards

## INTRODUCTION

Crops of the Brassicaceae family contain numerous phytochemicals that are known, or are suspected to be, beneficial for human health. These include sulfur-containing glucosinolates (GSLs) <sup>1</sup>, which have a range of hydrolysis products that are noted for beneficial effects on human health <sup>2</sup>. GSLs are secondary metabolites that are hydrolysed by myrosinases and modified by specifier proteins into numerous breakdown products <sup>3</sup>; these include isothiocyanates (ITCs), thiocyanates, nitriles, ascorbigens, indoles, oxazolidine-2-thiones and epithioalkanes <sup>4</sup>. This process is part of a complex defense strategy utilised by Brassicaceae plants to protect against herbivory, pests and diseases <sup>5</sup>. These compounds also give the family their distinctive sulphurous, hot, mustard and pepper flavors <sup>6</sup>.

Potential health benefits such as anti-carcinogenic and anti-metastatic activity have been linked with these compounds (such as ITCs and indoles) in cell and animal studies <sup>7</sup>. Clinical, epidemiological and pharmacological research in humans has demonstrated beneficial effects *in vivo* on some cancers, on cardiovascular health <sup>8,9</sup>, and on neurodegenerative prevention <sup>10</sup>. For these reasons, there is huge interest in enhancing Brassicaceae crop GSL content <sup>11</sup>. Despite initiatives such as the “5-a-day” campaign, fruit and veg consumption remains low in Western countries, and chronic diseases such as cancer and cardiovascular disease are leading to premature deaths <sup>12</sup>.

This review will explore prominent species and some underutilised edible Brassicaceae crops with the potential for GSL/ITC profile improvement. The health benefits that have been linked to these compounds and how they can be maximized will also be discussed. We aim to highlight and explore the challenges faced in developing enhanced Brassicaceae varieties in three key areas: plant breeding, agronomic practice, and ‘the consumer’. Previous review papers have not directly addressed the discrepancies between scientific research methods and common agricultural and commercial practices, or how plant

breeders can use scientific findings to inform their selections. Our goal is not to define the ideal crop for enhancement, but to highlight species that require further study and development. We encourage research groups to consider the entire commercial supply chain, and how this affects plant phytochemistry in a ‘real world’ context. We also highlight the need for consideration of the sensory preferences and end consumer metabolic genotypes. In this way, commercial breeders/producers can utilise better scientific research to improve crop nutritional density, and make informed decisions about varietal selection and agronomic practice.

## **BRASSICACEAE CROPS & GLUCOSINOLATE PROFILES**

### **General**

Table 1 summarises and compares the GSL profiles of several major, minor and underutilised Brassicaceae crops, and gives examples of typical concentrations that have been reported within the scientific literature. The following section describes these profiles and illustrates how concentrations and profiles vary between species.

### **Broccoli (*Brassica oleracea* var. *italica*)**

Perhaps the most well studied Brassicaceae crop is broccoli<sup>13</sup>. It is a well-known vegetable that is grown and consumed worldwide, and production rates are increasing<sup>14</sup>. The key factor in its popularity from a research perspective is that it contains significant glucoraphanin concentrations in florets and sprouts (Table 1)<sup>11,14–34</sup>. Total reported concentrations in broccoli florets are modest (~7.9 mg.g<sup>-1</sup> dw, Table 1) compared to other commonly consumed crops such as Brussels sprouts (~13.3 mg.g<sup>-1</sup> dw). That being said, some varieties have high total concentrations (26.9 mg.g<sup>-1</sup> dw<sup>16</sup>), well in excess of the average.

### **Brussels Sprouts (*Brassica oleracea* var. *gemmifera*)**

Although broccoli and kale are most often ascribed with the most potent health benefits associated with GSLs, Brussels sprouts have higher total concentrations than both, on average ( $\sim 13.3 \text{ mg.g}^{-1} \text{ dw}$ ). Although not containing high levels of glucoraphanin, sprouts do have high amounts of glucobrassicin (Table 1)<sup>18,19,25,31,32,34,35</sup>.

**Cabbage, Red Cabbage, & White Cabbage (*Brassica oleracea* var. *capitata*, *Brassica oleracea* var. *capitata* f. *rubra*, & *Brassica oleracea* var. *capitata* f. *alba*)**

Cabbage is a widely consumed and studied crop, but has modest total GSL concentrations compared to other crops (Table 1)<sup>1,25,31,32,36,37</sup>. White cabbage is similar to the green variety in terms of its overall GSL profile<sup>31,32,38</sup>.

Red cabbage contains similar GSLs to white and green cabbages, but differs in the relative amounts present within leaf tissues; e.g. it contains greater concentrations of glucoraphanin and gluconapin, and less sinigrin<sup>1,31,32,39–41</sup>. Overall, average reported concentrations are higher in red cabbages than other types.

**Cauliflower (*Brassica oleracea* var. *botrytis*)**

Total GSL reports in cauliflower florets range from  $0.7 - 11.4 \text{ mg.g}^{-1} \text{ dw}$ , but average  $\sim 4.1 \text{ mg.g}^{-1} \text{ dw}$ ; much lower than broccoli and Brussels sprouts. The predominant major GSL reported is glucobrassicin ( $\sim 1.7 \text{ mg.g}^{-1} \text{ dw}$ )<sup>18,19,21,22,25,31,32,34,42</sup>.

**Chinese Cabbage (*Brassica rapa* var. *chinensis* & *Brassica rapa* var. *pekinensis*)**

There are two predominant Chinese cabbage varieties: *B. oleracea* var. *pekinensis* and *B. oleracea* var. *chinensis*. These crops originate and are popular in China and southeast Asia, and have been identified as candidates for GSL accumulation trait improvement through breeding, due to large phenotypic variation<sup>43,44</sup>. Total average GSL contents reported are modest compared to other crops (Table 1). Indolic GSLs make up a large proportion of the overall profile<sup>31,32,36,43–45</sup>.

**Chinese Kale (*Brassica oleracea* var. *alboglabra*)**

Also known as gai lan, Chinese kale is a popular crop in China and southeast Asia, but not well known in other parts of the world. It is noted for high GSL concentrations (compared with broccoli florets). Total concentrations in mature leaves have been reported to be 14.9 mg.g<sup>-1</sup> dw<sup>21</sup> (broccoli florets: ~7.9 mg.g<sup>-1</sup> dw). In sprouts, GSL concentrations have been reported as high as 98.2 mg.g<sup>-1</sup> dw<sup>46</sup> and as low as 3.7 mg.g<sup>-1</sup> dw<sup>47</sup>.

#### **Collards (*Brassica oleracea* var. *sabellica*)**

Collards are an understudied variety of *B. oleracea*, but have high total GSL concentrations (18.2 mg.g<sup>-1</sup> dw). Sinigrin concentrations (6.5 mg.g<sup>-1</sup> dw), glucobrassicin (4.6 mg.g<sup>-1</sup> dw), progoitrin (2.9 mg.g<sup>-1</sup> dw) and glucoiberin (1.0 mg.g<sup>-1</sup> dw) make up the typical profile<sup>18,19</sup>.

#### **Ethiopian mustard (*Brassica carinata*)**

Ethiopian mustard is a traditional leafy crop of Africa and contains modest GSL concentrations. These include minor amounts of glucoalyssin, gluconapin, progoitrin, glucobrassicin, 4-hydroxyglucobrassicin, 4-methoxyglucobrassicin, neoglucobrassicin and gluconasturtiin, with the vast majority composed of sinigrin (Table 1)<sup>48</sup>. The crop is underutilised in terms of breeding and could be developed to a higher quality, both for human consumption and as a potential biofumigant crop<sup>49</sup>.

#### **Ezo-wasabi (*Cardamine fauriei*)**

Ezo-wasabi is a niche herb crop that originates from Hokkaido, Japan. It is a popular herb in this region and is characterised by a pungent wasabi-like flavor due to very high GSL concentrations. Abe et al.<sup>50</sup> identified three GSL compounds within leaves: glucoiberin, gluconapin and glucobrassicin. Total concentrations were reported to average 63.0 mg.g<sup>-1</sup> dw.

#### **Kale (*Brassica oleracea* var. *acephala*)**

Kale has been reported as having a wide range of health benefits, including those associated with GSLs<sup>51</sup>. Total concentrations are generally modest<sup>18,19,25,37,52</sup>, but some



studies report concentrations higher than broccoli. A comprehensive analysis of 153 field-grown cultivars by Cartea et al.<sup>37</sup>, found the average concentrations to be higher at 10.7 mg.g<sup>-1</sup> dw. The profile consists of predominantly aliphatic GSLs: with some aromatic and indole compounds present. The concentrations of the latter are reported as being highest, on average.

#### **Kohlrabi (*Brassica oleracea* var. *gongylodes*)**

Kohlrabi stems are low in GSLs with average concentrations amounting to ~2.2 mg.g<sup>-1</sup> dw. The profile is composed of glucoiberin, glucoraphanin, glucoalyssin, glucoiberin, glucoerucin, glucobrassicin, gluconasturtiin, and neoglucobrassicin, with some other trace GSLs identified<sup>18,19,31,32</sup>.

#### **Leaf rape & Turnip rape (*Brassica napus* var. *pabularia* & *Brassica napus*)**

Rapeseed leaves contain modest GSL amounts, but like collards are not widely consumed by the public. The bulk of the leaf rape GSL profile is made up of glucobrassicinapin, progoitrin and gluconapin<sup>53</sup>. Turnip rape by contrast is composed predominantly of gluconapin<sup>36</sup>. Sprouts have relatively high GSL abundances compared to the mature leaf tissue (Table 1)<sup>11</sup>.

#### **Maca (*Lepidium meyenii*)**

Maca roots are not commonly consumed in western diets, but are prominent in South American cuisine. Three main cultivar forms are consumed (red, purple, and black) and powders are popular as “food supplements” with anecdotal health benefits attributed to them. The species is an ideal candidate for improvement efforts, as it contains a wide variety of traits and compounds with purported health benefits, such as phytosterols<sup>54</sup>.

Total GSL concentrations are high relative to other root Brassicaceae with the primary compound being glucotropaeolin, and secondary glucolimnathin. This profile makes

the crop somewhat unique among Brassicaceae, with only glucoalyssin and glucosinalbin shared with more common cultivated species<sup>54</sup>.

### **Moringa (*Moringa oleifera*)**

Moringa species are non-cruciferous known for the high concentrations of aromatic GSLs found within tissues<sup>55</sup>, and the unusual multiglycosylated conformation of their structures. Within leaf tissues 4- $\alpha$ -rhamnopyranosyloxy-benzyl GSL (glucomoringin) is the dominant compound, with lower concentrations of acetyl-4- $\alpha$ -rhamnopyranosyloxy-benzyl, which exists in three isomeric forms (Ac-Isomer-GSLs I, II, III); these latter molecules each have an acetyl group at different positions on the rhamnose moiety. Due to the nature of these structures, standard methods of desulfation extraction are not recommended for moringa as artifacts are formed, which are not reflective of intact GSL analysis. A method for the stable extraction of these compounds has been developed by Förster et al.<sup>56</sup>. For this reason, papers utilising desulfation extraction in moringa should not be taken as representative of GSL profiles *in planta*.

The concentrations of GSLs reported for moringa leaves vary greatly (Table 1)<sup>57–60</sup>, due to diverse growing environments and cultivar choice. Stems and roots tend to have lower concentrations of glucomoringin and the acetyl isomers, but are noticeably higher than for more commonly consumed crops such as kohlrabi and rutabaga.

### **Mustard Greens (*Brassica juncea*)**

Like collards, mustard greens are high in GSLs (~25.9 mg.g<sup>-1</sup> dw), but not widely consumed due to their pungent and bitter tastes. Virtually all of the GSL profile is composed of sinigrin<sup>18,19,36</sup>. There are a large diversity of accessions and cultivars of this species, which provides an excellent resource for any breeding programs focused on culinary improvement.

### **Radish (*Raphanus sativus*)**

Radish encompasses several varieties such as ‘common’ radish, China Rose<sup>11</sup> radish, and Spanish black radish<sup>61</sup>. GSL concentrations reported from radish sprouts are very high<sup>11</sup> compared to some reports for roots<sup>31,32</sup>. There is special interest in the compound glucoraphasatin (also known as dehydroerucin) contained within radish tissues. It has been postulated that the cell detoxification properties of its ITC (4-methylthio-3-butenyl ITC; MIBITC) are comparable to sulforaphane (SFN)<sup>61</sup>.

**Rocket (*Eruca sativa*, *Diplotaxis tenuifolia*, *Diplotaxis muralis* & *Erucastrum* spp.)**

The rocket (rucola, arugula, roquette) species *Eruca sativa*, *Diplotaxis tenuifolia*, and *Diplotaxis muralis* are often grouped and classed together due to the similarity in GSL profiles. Other species, known as dogmustards (*Erucastrum* spp.), are also morphologically and phytochemically very similar to rocket.

Rocket species have five major GSL constituents: glucoraphanin, diglucothiobeinin, glucosativin, dimeric-glucosativin (DMB) and glucoerucin (Table 1)<sup>62–65</sup>. By comparison to broccoli, total average GSL concentrations are higher for rocket (*E. sativa*: ~15.3 mg.g<sup>-1</sup> dw; *D. tenuifolia*: ~11.2 mg.g<sup>-1</sup> dw), but average glucoraphanin concentrations are lower (*E. sativa*: ~2.0 mg.g<sup>-1</sup> dw, *D. tenuifolia*: ~1.7 mg.g<sup>-1</sup> dw).

Dogmustard and annual wall-rocket (*D. muralis*) profiles are somewhat different from ‘wild’ (*D. tenuifolia*) and ‘salad’ (*E. sativa*) species, but not as well studied. Dogmustard GSL profiles are low in total concentration, but much of this is glucoraphanin. Annual wall-rocket is high in this GSL too, by comparison to the commercially cultivated species, but few cultivars have been characterised to-date. It is also high in diglucothiobeinin, DMB and glucoerucin, giving a moderate total GSL concentration<sup>65</sup>.

The existence of dimeric GSLs in rocket species has proved controversial, with many papers accepting the hypothesis that they are products of extraction, without any supporting experimental evidence. Work by Cataldi et al.<sup>66</sup> a decade ago cast significant doubt on this

assumption, but has largely gone unnoticed within the literature. The addition of tris(2-carboxyethyl)phosphine (TCEP) to rocket extracts is common within the literature, and acts as a reducing agent to break disulfide bonds, such as those that exist in DMB and diglucothiobeinin. This so-called ‘prevention of artifact formation’ may actually be drastically modifying the GSL profile from its natural configuration. As is seen in *Moringa* spp., multiglycosylated GSLs do occur in nature, and so it is not inconceivable that these compounds are naturally synthesised. Little is known about rocket GSL biosynthesis beyond compounds common to other species (e.g. glucoraphanin and glucoerucin). The pathway for glucosativin, and indeed dimeric GSL, biosynthesis has yet to be elucidated<sup>67</sup>, and even less is known about their possible evolutionary and biological functions. In light of these unresolved questions dimeric compounds have been included in Table 1.

#### **Rutabaga (*Brassica oleracea* var. *rapifera*)**

Rutabaga (or swede) is consumed as a root crop and undergoes heavy processing and cooking before consumption (i.e. peeling, chopping & boiling) to soften the tissue. Raw GSL concentrations have been reported to range between 3.5 and 5.6 mg.g<sup>-1</sup> dw, with progoitrin reported as the most abundant GSL overall. The GSL profile is very diverse (Table 1), with concentrations being particularly high in sprouts<sup>31,32,68</sup>.

#### **Spider plant (*Cleome gynandra*)**

*C. gynandra* is known by several other common names, including: Shona cabbage, African cabbage, spiderwisp, chinsaga and stinkweed. It is a popular leafy vegetable in African traditional diets, and is routinely consumed for its purported medicinal properties. Despite this popularity, current cultivars perform poorly, making the species an ideal candidate for improvement<sup>69</sup>. Only one GSL is reported for spider plant, which is 3-hydroxypropyl (glucoerysimumhieracifolium; Table 1)<sup>70</sup>, and is most concentrated in the stems, siliques and flowers, with low leaf abundance<sup>69</sup>.

## **Watercress (*Nasturtium officinale*)**

Watercress is a crop that is gaining popularity in foods such as soups and smoothies, as well as a traditional garnish <sup>71</sup>. Like rocket, watercress cannot be considered domesticated due to a lack of breeding programs, and the tendency for commercial crops to be propagated through clonal cuttings rather than seeds <sup>6</sup>. Its GSL composition is made up almost entirely of gluconasturtiin. Its ITC is phenylethyl-ITC (PEITC) and is known to infer potential health benefits in humans <sup>6</sup>.

Small amounts of indolic GSLs are also found within tissues (Table 1) <sup>36,71</sup>, but few aliphatic GSLs have been reported. Total concentrations are modest ( $\sim 5.0 \text{ mg.g}^{-1} \text{ dw}$ ) but like rocket species, cooking is not essential before consumption.

## **White Mustard (*Sinapis alba*)**

White mustard leaves are not widely consumed due to their pungent attributes. They are high in GSLs, which is almost entirely made up of the aromatic GSL glucosinabin <sup>24</sup>. These crops are predominantly used as biofumigants to control soil borne pests, such as nematodes.

## **PLANT BREEDING**

### **General**

To quote Dr. Howard-Yana Shapiro, “It is not so much a question of more food. It is more a question of better food” <sup>72</sup>. This statement encapsulates the ethos of breeding Brassicaceae crops for enhanced GSL content. The trend in many crop breeding programs over the last 60 years has been to increase yield, but this has come at the expense of nutritional value in some instances <sup>73</sup>. It is hoped that by creating new and nutritionally dense varieties, development of chronic diseases such as heart disease, cancer, and dementia can be reduced through elevated concentrations in people’s diets.

Cereal crops have seen the greatest interest and investment in terms of genomics and breeding improvement over the last 150 years. It has been estimated that plant breeding has accounted for 58% of the increases in maize yields seen between 1930 and 1980<sup>74</sup>, and if the same concerted effort were to be made in Brassicaceae vegetables, it is not inconceivable that compounds related to health-benefits could also be improved.

As pointed out by Goldman<sup>75</sup>, the irony is that many of the most beneficial health compounds are being bred out of crops because they are also responsible for pungency and sensory traits which consumers dislike. But this could be remedied through breeding by also looking at corresponding ratios with free sugars, some amino acids, and the relative abundances of green-leaf volatiles. These have been shown to infer reductions in the perceptions of such traits, while maintaining GSL concentrations<sup>76</sup>.

The majority of genomic research for traits related to GSL metabolism has been conducted in species such as *A. thaliana*<sup>77</sup> and *B. oleracea*<sup>78</sup>. *De novo* genome sequencing costs are still high, but falling, and this may entice new exploration of minor Brassicaceae crop genomes in unprecedented fashion. There is however still a lack of understanding within the literature of how new Brassicaceae varieties are developed commercially through plant breeding methods. Such considerations are often absent from many nutritional, biochemical and medical studies<sup>79</sup>. Individuals who are skilled and adept at computational genomics, practical plant breeding, cultivation, analytical chemistry, and molecular biology techniques are scarce, and having a deep knowledge of these fields and how they each interact is challenging. This may be a reason why breeding efforts for phytochemical health traits to-date have lagged behind physiological traits as it requires interdisciplinarity, even when genomic information is available<sup>80</sup>. It is likely that in the private sector molecular breeding is already well established in some Brassicaceae crops, but the degree to which these efforts

have focused on GSL improvement are not readily apparent in commercial varieties available for human consumption.

A minority of people in Western countries consume an adequate amount of vegetables<sup>81</sup>, and even fewer are likely to consume the recently reported optimum of ten-per-day<sup>82</sup>. Breeders are recognising that getting consumers to eat more vegetables is not a realistic goal<sup>83</sup>. Instead, breeding strategies are concentrating on elevating compounds such as GSLs and ITCs through selection so that the vegetables on offer to the consumer have a higher nutritional density. A large proportion of people could benefit from resultant new varieties without having to modify their diets at all.

Much of the reported health effects are attributed to the GSL hydrolysis products of glucoraphanin, glucoerucin and glucobrassicin<sup>84</sup>, which could be increased through appropriate breeding selection. The ITC and indole products (SFN, erucin and indole-3-carbinol; I3C, respectively) have shown strong anti-carcinogenic effects in cell and animal studies<sup>85</sup>, but as will be discussed, these studies are limited in their applicability to humans and day-to-day consumption. There are many different factors that must be considered when breeding for modified GSL profiles. These will be discussed in the following sections; see Figure 1 for a summary.

### **Breeding For Increased Glucosinolate Content**

As highlighted within Table 1 there is huge scope for individual crop improvement, as evidenced by the diversity of GSLs and concentrations reported<sup>86</sup>. There are very few examples of successful stabilisation of GSL concentrations across environments however<sup>87</sup>. In order to develop enhanced varieties, species diversity must be scrutinised on a large number of cultivars/accessions before any breeding or genomics can take place<sup>79</sup>.

In *Arabidopsis thaliana* quantitative trait loci (QTLs), and the generation of robust single nucleotide polymorphism (SNP) markers have allowed detailed understanding of

298 numerous genotypes <sup>88</sup>. In order to develop such comparable resources for specific  
299 Brassicaceae crops, breeders and researchers must have a comprehensive and extensive  
300 knowledge of the cultivar breeding history, as well as a detailed knowledge of the GSL/ITC  
301 types produced across environments <sup>89</sup>. Due to the complexity of the *Brassica* genome and  
302 comparatively long life cycles of commercial crops, generating such genetic resources can  
303 take decades.

304 The GSL pathway itself in *Brassica* and *Arabidopsis* is now well elucidated <sup>90</sup> and it  
305 is possible to identify orthologous genes for biosynthesis, transcriptional regulation and  
306 environmental response in other species <sup>87</sup>. MYB transcription factors control the complete  
307 GSL biosynthetic pathway, and also influence primary and sulfate metabolic pathways.  
308 Differing transcript levels associated with MYB genes has been shown to affect indole GSL  
309 accumulation and the related metabolism products when plants are under pathogen stress <sup>91</sup>.

310 Aliphatic GSLs are synthesised from the amino acid methionine, and indolic GSLs  
311 from tryptophan <sup>92</sup>. The gene *BoGSL-PRO* in *B. oleracea* converts methionine into  
312 dihomomethionine and a chain-elongation process begins. This is further regulated by other  
313 genes such as *BoGSL-ELONG*, and determines the carbon side-chain length (e.g. propyl,  
314 butyl, pentyl, etc.). Other genes, such as *BoGSL-ALK*, further modify the R-group later in the  
315 synthesis pathway, and determine its final configuration <sup>77</sup>.

316 GSL biosynthesis levels are regulated by plant defense signaling compounds, such as  
317 salicylic acid (SA), ethylene and jasmonic acid (JA). The synergistic or antagonistic crosstalk  
318 between these three compounds determines the relative gene expression. Genes such as  
319 *CYP79B2*, *CYP79B3*, *CYP79F1* and *CYP79F2* regulate the GSL biosynthesis pathway and  
320 determine the overall GSL tissue profile, influencing the ratios between aliphatic and indolic  
321 GSLs <sup>93</sup>. The level to which these and other biosynthetic genes are expressed depends on the  
322 stimuli that initiate transcription, which can be both biotic and abiotic in nature. The



relationship with primary sulfur metabolism is also important for GSL production, as two to three sulfur atoms are required per aliphatic GSL molecule<sup>94</sup>.

The difficulty comes in generating breeding populations and having resources large enough to develop such detailed knowledge in non-model species. Some papers have advocated plant selection based on highest total GSL concentrations<sup>37,44</sup>, however this is an unsophisticated approach, as not all GSLs produce breakdown products which are beneficial for health, or positive for consumer acceptability. It also does not account for the potentially harmful effects of specific GSLs when ingested in large quantities.

The most comprehensive and thoroughly tested example of a crop bred for enhanced GSL content is *Beneforté* broccoli. This variety is an F<sub>1</sub> hybrid derived from an original cross between *B. oleracea* var. *italica* and *Brassica villosa* – a wild relative. The resultant variety is able to assimilate sulfur at an enhanced rate, but also allocate greater amounts to methionine-derived GSL production, rather than partitioned into the form of S-methylcysteine sulfoxide (SMCSO). SMCSO levels are reduced by an average of ~7% in plants containing the introgressed *B. villosa* *Myb28* allele, which in turn corresponds to a reciprocal increase in glucoraphanin<sup>23</sup>. Sarikamis et al.<sup>20</sup> also introgressed markers from *B. villosa* into broccoli which are associated with genes controlling the ratios between glucoraphanin and glucoiberin. Selection for such genes could influence the downstream health beneficial effects to the consumer.

Another area that could be targeted through breeding is hydrolysis product pathway modification. It is known for example that a gene in *A. thaliana* called *epithiospecifier modifier 1* (*ESM1*) encodes a protein that inhibits epithiospecifier protein (ESP) function, preventing it from converting GSLs into nitriles. Identifying, selecting and breeding for such genes in Brassicaceae crops would be instrumental for improving the predictability of hydrolysis product formation. Nitriles are much less bioactive than ITCs, and it would be

favourable to decrease production of them <sup>95</sup>. This would lead to increases in ITC abundance and enhance potential health benefits. Selecting for GSL accumulations alone is therefore not sufficient to produce enhanced varieties; ITC abundance ratios must also be considered, as these vary between species, varieties and genotypes <sup>96</sup>.

The variability of GSL concentrations in crops is due to genetic responses which are influenced by environmental interactions <sup>17</sup>. The specific mechanisms responsible for such large variations seen in varieties are complex <sup>97</sup>, and are not well understood in the commercial supply chain context. Few research papers have replicated the food system to determine the effects on GSL and hydrolysis product concentrations from a plant breeding perspective <sup>98</sup>, and so it is difficult to make informed selections.

If products like *Beneforté* are to be developed for other species, it will require screening a large number of germplasm accessions in multiple environments, and phytochemical analysis throughout the commercial food chain <sup>86</sup>. Gene bank accessions are an underutilised resource for breeding enhanced GSL accumulation traits. Screening these large collections for enhanced traits is challenging, but wild genotypes with enhanced glucoraphanin, glucoerucin, glucoraphenin, glucoraphasatin, glucoiberin, sinigrin and indole GSLs may be found <sup>37</sup>. Blueprint breeding schemes for this method of introgression already exist <sup>20</sup> and so it is feasible that other crops could be developed with enough time and resources.

Developing the genomic tools to improve varieties will also be necessary in future. Despite detailed knowledge of the *Arabidopsis* and *Brassica* genomes there are few other related crops that have been sequenced. Developing analogous genetic markers, linkage and QTL maps using these species will serve for a time to screen for common GSL traits; however, species such as rocket, radish and watercress have very different GSL profiles to *B. oleracea* and *Arabidopsis*. As such, the time will come when full genome sequences will be

required for these crops, to develop and enhance GSLs/ITCs with a high level of precision<sup>80</sup>. Having species specific SNPs associated with GSL/ITC QTLs, genes, transcription factors, and other plant defense and senescence pathways will be a powerful tool for enhancing crops, and significantly reduce the generation number required to develop new breeding lines and varieties<sup>89</sup>.

### **Breeding For Decreased Glucosinolate Content**

From the late 1960s to the mid-1990s, much of the focus on GSLs and the associated hydrolysis products was in relation to adverse health effects. There was concern surrounding goitrogenic compounds, which are produced from the GSLs epiprogoitrin and progoitrin. The oxazolidine-2-thiones and thiocyanate compounds produced by the hydrolysis of these GSLs interfere with thyroid metabolism and induce a condition known as goiter. In the presence of nitrate they also undergo nitrosation reactions, which is thought to have negative health consequences<sup>99</sup>. High doses of GSL-derived nitriles have also been shown to be toxic<sup>100</sup> but reports are conflicting<sup>101</sup>. This has led to arguments for decreasing certain GSL compounds in Brassicaceae crops through selective breeding. Progoitrin, sinigrin, gluconapin and indole GSLs have all been cited as contributors to bitterness<sup>87</sup>, and a reduction is thought to improve consumer acceptance<sup>102</sup>.

Sinigrin is common (in low concentrations) in important crops, such as cabbage, kale, broccoli and Brussels sprouts (Table 1). The relative abundances in these are minor compared to those found in mustard greens (~16.6 mg.g<sup>-1</sup> dw), Chinese kale sprouts (~8.4 mg.g<sup>-1</sup> dw) and collards (6.5 mg.g<sup>-1</sup> dw)<sup>18,19</sup>. The reduced bitter compound concentrations in commercial crops have led some to speculate if this is partly the reason why pesticides have to be used so intensely, as these varieties may be more prone to disease and herbivory<sup>102</sup>.

There are opposing opinions relating to sinigrin concentrations within Brassicaceae foods. Sensory analysts advocate its reduction, as it is “*regarded not as a health benefit but*

as a major sensory defect”<sup>102</sup>. Other studies by contrast have argued that sinigrin should be increased due to the associated health benefits of allyl-ITC (AITC)<sup>37</sup>. Opinions expressed in sensory quality reviews perhaps do not appreciate how difficult ‘removal’ is from a breeding perspective, or what the effects are from a pest and disease management standpoint. These compounds do not exist simply for the pleasure or displeasure of the human species. It perhaps demonstrates a misunderstanding of the endogenous function of such compounds within plants, and ignores any health benefits they have.

Progoitrin has been found to be prevalent in Chinese kale sprouts (~14.8 mg.g<sup>-1</sup> dw), collards (2.9 mg.g<sup>-1</sup> dw<sup>18,19</sup>), and leaf rape (2.2 mg.g<sup>-1</sup> dw<sup>53</sup>; Table 1). Arguments have been made for progoitrin reduction in commercial crops because of the association between its degradation products and goiter<sup>87</sup>. Double recessive alleles of GSL biosynthesis genes have been identified and utilized in reducing concentrations in rapeseed to improve livestock feed<sup>90</sup>. Similar efforts to reduce harmful GSLs in other Brassicaceae is a realistic goal, but must be targeted so that beneficial GSL accumulation is not affected.

Most arguments for the goitrogenic effects of GSLs are outdated and unsupported in humans, however. Not all GSLs have goitrogenic breakdown products, and so are unlikely to adversely affect otherwise healthy humans<sup>103</sup>. Most cited evidence stems from studies in herbivores, such as rabbits and cows, which can ingest large amounts of seed meal and leaves a day<sup>104,105</sup>. Assuming humans who eat Brassicaceae vegetables don’t have a severe pre-existing thyroid condition, and are not suffering iodine deficiency, there is little evidence of healthy people developing goiter through ingestion of leaves, sprouts, roots, or indeed the milk of animals that consume large GSL quantities<sup>103</sup>. At low-moderate levels the compounds are beneficial to humans and enhance cellular defenses against cancer and other diseases<sup>106</sup>.

## 423 **CULTIVATION, POSTHARVEST PROCESSING & STORAGE**

### 424 **General**

425         Improved genetics and phytochemical content through breeding must be synergistic  
426 with improvements in Brassicaceae agronomy and cultivation methods. Important aspects to  
427 be considered when attempting to enhance GSL concentrations through breeding include:  
428 appropriate varietal selection, responses to fertilizer application, water availability, harvest  
429 time/growth stage, light levels, and local temperature <sup>107–112</sup>. These factors and many more  
430 can have a significant impact on the quantities of GSLs produced by plants (see Table 2). It  
431 has been reported that GSLs can be enhanced through better and more informed cultivation  
432 methods by up to ten times in the case of broccoli and cauliflower, and doubled in radish <sup>86</sup>.

### 433 **Varietal Selection**

434         It is well documented that GSLs and the respective breakdown products vary between  
435 species, within species, and even within individual cultivars <sup>86</sup>. The data collated in Table 1  
436 gives examples of this variability, with large concentration ranges reported for species  
437 according to different growing environments (e.g. field or glasshouse).

438         It has been reported that a high degree of variation in GSL concentrations can exist  
439 between plants of the same variety (e.g. in *Marathon* broccoli heads) <sup>113</sup>. This poses a  
440 significant challenge, especially if varieties are uniform hybrids for morphological traits; and  
441 indicates just how great an impact environment has upon GSL accumulation. In experimental  
442 terms, it has been suggested that replicates be increased or samples pooled to create a  
443 ‘representative’ picture <sup>113</sup>. This is perhaps a neater approach statistically, but obscures the  
444 inherent variation present between plants of the same variety, giving a false sense of  
445 uniformity. If plants have not been selected for GSL profile modification, it is unsurprising  
446 that such high variations exist <sup>96</sup>; therefore the development of uniform breeding lines and  
447 varieties will mitigate this by considering individual plant chemotypes and sensotypes.

## Light Intensity

It has been demonstrated in *A. thaliana* that UV-B radiation can induce gene expression that promotes GSL accumulation<sup>114</sup>. In crops such as broccoli and cauliflower it has also been observed that increased light levels can increase glucoraphanin and glucoiberin concentrations within florets<sup>86,115</sup>. In an excellent recent paper by Moreira-Rodríguez et al.<sup>116</sup> it was demonstrated that 24 hours after exposure to high UVB treatment, broccoli sprouts showed large increases in GSL concentrations. This included a 73% increase in glucoraphanin, 78% increase in glucobrassicin, and a 170% increase in 4-methoxyglucobrassicin. The authors indicated that UVB radiation triggers signal transduction pathways, leading to up-regulation of GSL biosynthesis genes as part of a UV protection mechanism. Within a segregating population of plants, it is theoretically possible to select for plant with genes predisposing them for such higher accumulations. With more advanced genetic analysis of such genes, it should also be possible to identify polymorphisms underlying the propensity for increased glucoraphanin and indole GSL biosynthesis. As the authors discuss, it may be theoretically possible to ‘tailor’ GSL profiles to a degree, by exposing sprouts to differing combinations of UVA and UVB light intensities. As with most studies of this kind, only a single variety of broccoli was used, and so it is not possible to determine how much these responses vary according to genotype. It was also not determined how these respective increases affected ITC/nitrile/indole production. Other studies have noted that GSL profiles are not necessarily indicative of myrosinase activity or hydrolysis product profiles<sup>110</sup>. Nevertheless, the results indicate that this is an area for future study, and it would be intriguing to determine how such responses vary within segregating populations of broccoli and other Brassicaceae.

GSL accumulation is generally much higher when plants are exposed to longer periods of light. A study by Kim et al.<sup>117</sup> showed that GSL concentrations of Chinese

cabbage seedlings were up to 6.9 times higher in plants exposed to light ten days after sowing. This suggests that raising seedlings in the dark for several days may increase the potential accumulations within the plants at later developmental stages.

GSL concentrations also fluctuate according to diurnal rhythms imposed by exposure to light and dark. Huseby et al.<sup>118</sup> demonstrated that relative expression of genes associated with GSL biosynthesis in *A. thaliana* were significantly increased in plants grown in dark conditions before being exposed to light, compared with those which were only exposed to a normal diurnal cycle. This implies not only that GSL biosynthesis can be influenced by light, but also that GSL concentrations can be enhanced through controlled exposure. Huseby et al. also saw GSL concentrations peak eight hours after light exposure was initiated in a diurnal cycle, with concentrations then subsequently declining. This has large implications for commercial operations that may harvest at specific times during the day. More research is needed to understand how these mechanisms function in commercial crops, but it is likely that recommendations for optimum harvest times could be generated in order to maximise GSLs.

Different light wavelengths that are applied to Brassicaceae crops also cause differing effects on GSL concentrations. Blue light has been shown to increase total GSLs in ezo-wasabi leaves<sup>50</sup> and turnip roots<sup>119</sup> (Table 2) via possible activation of GSL biosynthesis enzymes. This mechanism has been postulated but not verified, and is thought to impact aliphatic and aromatic GSLs, not indolic, as there is no corresponding increase for these compounds under blue light<sup>120</sup>. This phenomenon could be exploited in controlled environment cultivation or vertical farming methods, to improve the nutritive value of niche microleaf and baby leaf crops. In contrast, increased levels of red and far-red light have resulted in elevations of gluconasturtiin in watercress. It has also been reported that red light

(640 nm) applied to kale sprouts increases the production of specific GSLs, such as sinigrin; but other wavelengths have no significant effect<sup>107</sup>.

### **Environmental Temperature**

Unlike light intensity, increasing temperature does not have a reciprocal effect on GSL concentrations. Myrosinase activity is known to increase with higher daily mean temperature, and it is hypothesised that this leads to increased GSL degradation upon harvest<sup>86</sup>. It has been noted that high summer field temperatures have a detrimental effect on specific GSL concentrations at the point of commercial harvest in ‘salad’ rocket, but this is not indicative of postharvest concentrations, which have been observed to increase during shelf life storage<sup>98</sup>.

There are reports of increasing GSL concentrations with warmer weather in kale and red cabbage<sup>1</sup>, but these come from spring and autumn comparisons where differences in light levels may contribute more to the elevations observed than the relative increase/decrease in temperature. Steindal et al.<sup>52</sup> found a specific increase of sinigrin in kale at low growing temperatures. Schonhof et al.<sup>121</sup> analysed broccoli at different growth temperatures and found that low temperatures increased aliphatic GSLs, and high temperatures increased indolic GSLs. This trend was not observed by Steindal et al.<sup>52</sup> in kale, where both high and low temperatures (32°C & 12°C) increased aliphatic GSLs. The authors suggested that cold temperature stress is beneficial for GSL accumulation, but is dependent on the organs and species in question.

### **Water Availability**

In broccoli plants it has been observed that a reduction in water availability causes large increases in GSL concentrations<sup>86</sup>. This may be due to a concentration effect within the plant tissues, but it is also possible that this is a defensive response in times of vulnerability and stress. Various reasons have been hypothesised for such increases when plants are



experiencing drought, including increased synthesis of sugars, amino acids, and sulfur availability<sup>107</sup>.

As with other abiotic factors influencing GSL concentrations, there are conflicting reports. Some studies suggest that increased rainfall in the spring (coupled with increasing temperatures) increases GSLs<sup>1</sup>; but these interacting factors, combined with lengthening days and stronger light might be the primary cause. The timing of irrigation before harvest also impacts the abundance of GSLs, and is another factor for consideration<sup>107</sup>.

### **Sulfur Application**

Fertilizer application to Brassicaceae crops is common practice in the commercial setting but can lead to changes in GSL composition. High sulfur doses applied to crops can facilitate sizeable increases in GSLs with known health benefits (Table 2) such as glucoraphanin<sup>23</sup>. Application to broccoli plants (600 mg S plant<sup>-1</sup><sup>86</sup>) has been shown to increase concentrations. Combined with a reduction in watering, this can also boost the concentration, but at the sacrifice of yield<sup>86</sup>. Fertilizer cost may be a limiting factor for many growers, however. So while sulfur application to enhance GSLs may be effective, farmers will not be likely to adopt it unless yields can be maintained.

In radish, a lower amount of sulfur has been reported to be efficacious in increasing glucoraphasatin concentrations (150 mg S plant<sup>-1</sup>)<sup>86</sup>, meaning that application on specific crops could be more preferable and affordable from a commercial perspective. Increases in total GSLs, sinigrin, glucobrassicinapin, gluconapin and progoitrin have also been reported with increased sulfur<sup>107</sup>. For an excellent review of sulfur assimilation, its relationship with GSL biosynthesis, and the underlying genetic mechanisms responsible in *Brassica* species, see Borpatragohain et al.<sup>122</sup>.

### **Nitrogen Application**

With decreasing nitrogen application GSLs have been observed to increase<sup>86</sup>. In combination with sulfur fertilization (60 kg.ha<sup>-1</sup>), increasing nitrogen (80 – 320 kg.ha<sup>-1</sup>) has been shown to be ineffective at increasing total GSL concentrations in turnip, but can shift the ratio towards greater indolic GSL production. This is in contrast with sulfur applications at a low level (10 – 20 kg.ha<sup>-1</sup>) and increasing nitrogen, where aromatic and aliphatic GSLs decrease<sup>123</sup>.

Experiments by Schonhof et al.<sup>124</sup> in broccoli found that inadequate nitrogen increased GSLs, and inadequate sulfur decreased them. Hirai et al.<sup>125</sup> found that under nitrogen and/or sulfur limited growth conditions in *A. thaliana*, the genes encoding myrosinase enzymes were down-regulated in order to facilitate GSL storage in leaf tissues. The strategy for fertilizing commercial Brassicaceae crops should therefore take these factors into account if enhanced health properties are to be produced.

### **Methionine Application**

Another means of increasing GSL concentration in crops is amino acid application (Table 2). As aliphatic GSLs (such as glucoraphanin) are derived from methionine, application to crops could enhance production in species such as broccoli<sup>86</sup>. It has been applied to broccoli sprouts and rutabaga with encouraging results. In these crops, total GSLs were increased by 19% and 85%, respectively<sup>11</sup>. The effects on glucoraphanin and glucoiberin in the broccoli sprouts were modest, with a 7% increase. By contrast, indolic GSLs 4-hydroxyglucobrassicin, glucobrassicin and 4-methoxyglucobrassicin increased by 28%. In the rutabaga the large total increase was due to elevations in both aliphatic and indolic GSLs.

Baenas et al.<sup>11</sup> have suggested that the effects are strongest at lower concentrations (5 – 10 mM applications) which result in total GSL increases of 21 – 23% in sprouts. Other studies have applied up to 200 mM of methionine and still seen increases of up to 28%<sup>126</sup>,

though the application method was different. The effects on specific GSLs in sprouts related to health benefits such as glucoraphanin, glucoraphenin and glucoraphasatin seem not to be affected by methionine application according to Baenas et al.<sup>11</sup>, but this may be related to the immature growth stage at which plants were tested.

### **Selenium Application**

Selenium is an essential micronutrient for humans. There is a significant relationship between the amount of selenium within the diet and the risk of developing conditions such as cancer, heart-disease and immune system diseases<sup>127</sup>. It has been estimated that 33% of children (age 11-18), 39% of adults (age 19-64), and 44% of older adults (age 65+) consume less selenium than the recommended Lower Reference Nutrient Intake (LRNI) recommendation<sup>128</sup>.

Research has been conducted to apply selenium to crops (such as broccoli) to enhance nutritional properties<sup>129</sup>. Studies have shown that excess selenium application can reduce GSL content by 90%<sup>30</sup>. By contrast, selenium application to radish plants has been shown to increase glucoraphanin concentrations within roots<sup>129</sup>. With more moderate application, SFN concentrations can be increased in broccoli<sup>130</sup>, but other studies have reported no change in sprouts, indicating the optimum benefits of application depend on growth stage<sup>127</sup>.

### **Plant-Bacterial Interaction**

In a 2009 paper, Schreiner et al.<sup>36</sup> demonstrated that an auxin-producing bacterial strain (*Enterobacter radicicitans* DSM 16656) could influence and utilise GSL concentrations in several Brassicaceae species. The bacterial strain colonized the plant phyllosphere, and it was hypothesised that the response could be two-fold: 1) that GSL concentrations increased due to defense mechanism activation, and 2) that the bacterial auxin supply to leaves could induce GSL synthesis by metabolism of indole-3-acetaldoxime. The species with the greatest bacterial growth of *E. radicicitans in vitro* had high aliphatic GSL

concentrations (*B. rapa* & *B. rapa* var. *chinensis*), whereas aromatic GSL-containing species showed little increase (*N. officinale*).

Very few papers have linked bacterial colonisation of leaves with GSL accumulation, but Bell et al. 2017<sup>98</sup> found strong correlations between GSL concentration and bacterial load of rocket within the commercial supply chain after processing. This could be suggestive of defensive responses due to damage incurred through processing, but also that bacteria influence the GSL profile in some way during shelf life. This is an area of research that requires much more thorough exploration.

#### **Developmental Stage (Ontogeny)**

The developmental stage (ontogeny) at which plants are harvested is a significant determining factor in the GSL concentrations that will be ingested by consumers<sup>37</sup>. Crop maturity from a culinary perspective does not always coincide with peak GSL accumulation, as this can vary over life cycle. In broccoli heads, the highest glucoraphanin concentrations have been observed at 180 days after sowing, with a subsequent decline at the onset of flowering<sup>14</sup>. In contrast, Chinese kale GSL concentrations are reported to peak at the sprout growth stage<sup>47</sup>.

Sprouts are often the subjects of environmental, elicitation and postharvest studies to increase GSL accumulation<sup>47</sup>. This is because of the fast turnaround times in which crops of such age can be sown and harvested, and because it has been reported that GSLs are of higher concentration at this point. This is thought to be due to a concentration effect as leaves are not fully expanded, and therefore not diluted by growth and expansion<sup>11</sup>. Broccoli, cauliflower and cabbage studies have shown that total aliphatic GSL concentrations decline during a seven day sprouting period, but that indolic GSLs increased<sup>107</sup>. This is a very short space of time compared to the entire plant life cycle, and not representative of peak accumulation. Baenas et al.<sup>11</sup> specified that eight-day-old sprouts were optimum for

enhancing GSL concentrations, broccoli, turnip, rutabaga, and radish all much higher than their average reported mature values. They reported broccoli glucoraphanin concentrations of 18.3 mg.g<sup>-1</sup> dw. China Rose radish sprouts are especially rich in glucoraphenin and glucoraphasatin, and rutabaga high in progoitrin. Qian et al.<sup>46</sup> reported total concentrations as high as 98.2 mg.g<sup>-1</sup> dw in Chinese kale (grown hydroponically). It may be that sprout concentrations vary between species and varieties, and this needs to be addressed by analysing multiple commercial varieties and wild cultivars of each species. Sprout consumption is an uncommon practice for the consumer at the present time, so research in the mature crop may be of more relevance for enhancing GSL intake. That being said there is little consensus on what the best harvest point is to maximise GSL concentrations for individual crops, or even commercial varieties. As pointed out by Bell et al.<sup>62</sup>, some studies analysing the GSL composition of mature rocket leaves are often long after a commercially relevant time point, and so this needs to be addressed with consideration for common commercial practices.

An excellent paper published recently by Hanschen & Schreiner<sup>110</sup> explored the effects of ontogeny upon GSL and ITC concentrations in broccoli, cauliflower, cabbage, savoy cabbage, and red cabbage sprouts and heads. Importantly, they also tested multiple varieties for each crop, highlighting how important this is as a consideration for enhancing health-promoting compounds. It was observed that both the types and concentrations of GSLs and hydrolysis products differed between sprouts and heads, with up to ten times more present in the former than the latter. It was also apparent that for the tested varieties nitriles were the predominant hydrolysis product, indicating that this is an area for potential improvement through selection of genes related to ITC-nitrile ratios. The authors also pointed out that ‘mini heads’ contained the greatest concentrations of ITCs (such as sulforaphane), and are perhaps a better alternative to fully mature heads in terms of maximizing ITC

consumption. The only drawback of the study was that the reported concentrations were for raw plant material, not cooked. As discussed in the following ‘Consumer’ section, this may have drastic effects upon myrosinases and ESP proteins, and determining the amounts and types of hydrolysis products present at the point of ingestion.

In watercress, a crop which does not require cooking, an ontogenic study by Palaniswamy et al.<sup>131</sup> showed that leaves harvested at 40 days of growth after transplantation contained 150% higher PEITC than leaves at 0 days. This was a linear increase with no significant changes at 50 and 60 days. In species such as watercress where establishment of new breeding programs and varieties is difficult (due to the commercial preference of vegetative propagation), the selection of an optimum harvest date may be the most effective way in the short-term to promote maximum ITC formation in commercial crops.

### **Postharvest Commercial Processing & Storage**

It is well known that GSL profiles change during postharvest processing and storage. Processing can alter food matrix composition, which increases the accessibility and bioavailability of compounds<sup>34</sup> such as ITCs. The atmosphere in which produce is stored also affects GSL concentrations<sup>13</sup>.

In rocket species simulated shelf life storage has revealed that individual GSLs such as diglucothiobeinin increase<sup>63</sup>. After harvest and commercial processing significant increases in glucosativin and SFN have been observed. This indicates that postharvest industrial practices induce GSL synthesis and may boost the health beneficial effects for the consumer<sup>98</sup>. Glucoraphanin has likewise been shown to increase<sup>63</sup> or remain stable<sup>98</sup> throughout cold storage conditions, and the increases in ITCs over nitriles during storage has also been documented<sup>96</sup>. These results are encouraging, as it was previously assumed that concentrations would be detrimentally affected by rigorous harvest and washing procedures.

These trends have also been reported in broccoli, where total GSLs have been shown to increase by up to 42%, but at high storage temperature (10°C)<sup>132</sup>. It has been suggested that increases in glucoraphanin are due to the vegetative state of the broccoli heads<sup>14</sup>. At cold-chain temperatures (0-4°C) results are more conflicting; Rybarczyk-Plonska et al.<sup>14</sup> reported no changes in GSL concentrations, Fernández-León et al.<sup>133</sup> reported increases in aliphatic GSLs and decreases in indole, and Rodrigues & Rosa<sup>134</sup> saw stable indole GSLs, but a 31% reduction in glucoraphanin.

When combined with the addition of low postharvest light (13-25  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) at 10°C and 4°C, aliphatic GSL concentrations have been observed to increase by up to 130%, with 4-methoxyglucobrassicin also increasing<sup>14</sup>. It is unclear if the shift to warmer temperature during storage has any implication for tissue degradation or increased microbial load. These increases are arguably the result of stress responses due to the shifts in temperature from 0°C<sup>14</sup>, with the relative increases seen are dependent upon dose, frequency, and duration of UV-B exposure<sup>135</sup>. Increases have been reported for 4-hydroxyglucobrassicin at 18°C with 25  $\mu\text{mol m}^{-2} \text{s}^{-1}$  light<sup>14</sup>, but it is difficult to see how these recommendations can be applied to commercial produce.

## THE CONSUMER

### General

Some consumers are becoming more health conscious, and while not always the primary decision in purchasing and eating food, nutritional content is an aspect which is more evident in the decision-making process<sup>86</sup>. They are looking for products that are “healthy” and “natural”, and scrutinizing the nutritional value of Brassicaceae crops<sup>136,137</sup>. This is especially the case for young consumers, who are open to trying new foods<sup>138</sup>. That being said, the average contribution to the “five-a-day” that Brassicaceae account for is between 0.2

– 0.5 servings <sup>137</sup>, and even further from the optimum “ten-a-day” <sup>82</sup>. This section will explore the processes relating directly to the consumer after purchase, such as cooking, sensory perceptions and preferences, and human health and metabolic aspects.

Previous reviews have addressed the mechanisms involved in processing and the changes initiated in GSL and ITC profiles <sup>2,139</sup>. Few however have done so with the purpose of using such data to inform plant-breeding selections and improving the varieties themselves, rather than the methods used to process them. The effects of cooking on ITC formation in one variety of cabbage may not be the same as another, for example. The taste of one rocket variety may be preferred over another because of underlying phytochemical interactions with ITCs. The relative stability of myrosinases between broccoli varieties may determine the formation of ITCs over nitriles. All of these are quantifiable traits that can be used to inform breeding selections, and can be linked to the biochemistry and physiology of plants, which are ultimately determined at the genetic level.

### **Cooking Methods**

The means by which produce is prepared by the consumer influences the amounts of beneficial compounds that are ingested <sup>140</sup>. This includes all aspects relating to peeling, chopping and cooking. Depending on the species, this affects GSL concentrations and the production of hydrolysis products that are responsible for health benefits.

The heat generated by cooking often leads to myrosinase inactivation at temperatures  $>60^{\circ}\text{C}$  <sup>18</sup>, and is a barrier to increasing health benefits. In addition to this, high temperatures ( $\geq 100^{\circ}\text{C}$ ) also cause GSL degradation when tissue water content is  $>34\%$  <sup>33</sup>; this means commercial produce would be severely affected. Boiling crops like watercress results in severe GSL losses – probably through such thermal degradation <sup>71</sup>.

Steaming of vegetables has produced some conflicting results. Papers have reported GSL losses, some no-significant change, and others have observed significant increases <sup>140</sup>. A



study by Giallourou et al.<sup>71</sup> on the effects of cooking on watercress, found that steaming significantly increased gluconasturtiin concentrations (from 1.8 to 2.0 mg.g<sup>-1</sup> dw), and Gliszczynska-Świgło et al.<sup>141</sup> reported a 1.2-fold increase in total GSLs in broccoli. In the latter study, the authors hypothesised that this increase was time dependent, having seen no significant effects before 3.5 minutes of steaming. Similarly with watercress, steaming for 2-5 minutes saw no major losses in GSLs. This suggests there is an optimum time to steam in order to increase or preserve GSL bioavailability and avoid their breakdown due to prolonged heat. Another study looking at broccoli steaming found an increase in total GSL content<sup>141</sup>, however it is speculated that this is because cooking and heating increases compound extractability<sup>33</sup>. This translates into greater bioavailability and benefits to the consumer<sup>30</sup>, and it has been demonstrated in simulated *in vitro* digestion of cauliflower that sinigrin bioavailability is increased by 29.5% and 114.7% after steaming and boiling, respectively<sup>142</sup>. Ciska & Kozłowska<sup>143</sup> hypothesised that the disintegration of tissues by heat releases GSLs which would otherwise be bound within cell walls; this would account for the relative increases observed. But GSL bioavailability is of little significance for human health unless there is a means by which they can be hydrolysed into ITCs/indoles.

Microwaving has been found to induce severe GSL losses in numerous studies. As with steaming, it has been hypothesised that microwaves cause a cell structure collapse leading to contact between GSLs and myrosinase<sup>140</sup>. No studies have determined if there is a respective increase in ITCs as a result, or whether myrosinase is inactivated due to high temperatures.

Matusheski et al.<sup>144</sup> have demonstrated that cooking chopped broccoli heads at 60°C for 5 – 10 minutes increases and favors SFN production. It was hypothesised that the 60°C heat inactivated ESPs leaving myrosinase active and free to convert GSLs to ITCs. Such optimization methods for maximizing content signify that high SFN concentrations could be

ingested even after cooking, providing that heating is not too prolonged or intense. Breeding efforts should therefore focus on selecting plant lines with greater myrosinase function and stability at higher temperatures.

### **Condiment Selection**

There is some evidence to suggest that the condiment with which Brassicaceae are ingested aids in ITC production and enhances absorption within the gastrointestinal tract (studied in rats). Ippoushi et al.<sup>145</sup> have demonstrated that when raw, grated daikon radish is prepared in oil, the ITC absorptive content was increased compared to water. This perhaps suggests that oil stabilizes and preserves ITCs before ingestion.

The addition of exogenous myrosinase to cooked Brassicaceae has also been suggested as a means to boost GSL conversion to ITCs<sup>18</sup>. This commonly means the addition of mustard to foods, but many people find the pungency of this condiment too intense.

### **Sensory Perceptions**

The effects of differing GSL content in produce on the consumer and their tastes are very complicated<sup>68</sup>. It is known that not all consumers are the same in their preferences for Brassicaceae vegetables due to differences in genotype and life experience<sup>146</sup>. Certain GSLs and their hydrolysis products have been attributed with bitter tastes. The rejection of bitter tastes by some consumers is a barrier to encouraging greater consumption<sup>13</sup>, especially if breeding goals are to increase quantities within tissues<sup>102</sup>. It has been demonstrated that bitterness perceptions can be reduced or even masked<sup>147</sup> by enhancing relative sugar concentrations within tissues<sup>146</sup>. Therefore, through selective breeding, health-beneficial bitter compounds can be enhanced without negatively impacting on consumer acceptance.

Crop sensory improvement through plant breeding is perhaps even further behind efforts to breed for health benefits. These two should go hand-in-glove, but often are not considered together in published research papers. The trends seen in consumers preferring to

purchase more nutritious foods has not been mirrored by an improvement of the sensory properties of the foods themselves<sup>148</sup>. This means that if this trend is to be expanded or sustained, new varieties will need to be produced with enhanced sensory and nutritional traits, not just one or the other.

#### **Gut Microflora**

Many cooking studies on Brassicaceae have reported significant increases in available GSLs, but often omit that the temperatures involved would significantly or completely inactivate myrosinases. This means that any GSL to ITC and indole conversion would be reliant upon gut microflora. Some bacteria found within the human gut are known to possess myrosinase-like enzymes. They act as a potential means by which humans can ingest ITCs, even if cooking has inactivated plant myrosinase. It has been speculated that such bacteria play a vital role in mediating the health benefits of ITCs, but the degree to which this occurs is unclear and requires extensive study<sup>106</sup>.

#### **Consumer Health Benefits – Evidence From Cell & Animal Studies**

The vast majority of knowledge accumulated around ITCs comes from cell and animal studies. ITCs and indoles are classed as anticarcinogens and act as blocking agents that increase cytochrome P450 activity<sup>149</sup>; see Figure 2 for chemical structures of the most widely studied compounds. The prevailing mechanism of action suggested within studies is phase II metabolic detoxification enzyme activation, such as glutathione-*S*-transferase (GST), NAD(P)H:quinone oxidoreductase (NQO), and phase I enzyme inhibition<sup>149–151</sup>. Waste metabolites produced by cells are excreted into the blood and converted by the liver into mercapturic acid; this is then excreted in the urine<sup>96</sup>.

SFN has been linked with detoxification pathway modification, which increases the excretion of potential carcinogens from cells<sup>30</sup>. It is also linked with prostate cancer cell apoptosis, and has been shown to act in a dose-dependent manner against kidney and

795 colorectal cancer cell lines by inhibiting histone deacetylation <sup>150</sup>. There is also evidence to  
796 suggest that the increase in phase II detoxification enzymes by SFN could help reduce  
797 damaging effects in basal ganglia, and protect dopaminergic neurons <sup>10</sup>; this has significant  
798 implications for neurodegenerative diseases. For an excellent review of the neuroprotective  
799 effects of SFN see Giacoppo et al. <sup>10</sup>.

800 ITCs such as PEITC (abundant in watercress) and AITC (abundant in mustards) have  
801 been shown in cell studies to inhibit tumorigenesis, protect DNA from damage, and induce  
802 apoptosis. The specific structure and length of the alkyl chain an ITC has is linked to its  
803 efficacy in inhibiting tumor formation. Phenylhexyl ITC (C<sub>6</sub>; PHITC) is 50 – 100 times more  
804 efficacious in this respect than PEITC <sup>150</sup> in studies focused on reducing the effects of  
805 smoking. The dose used however was 5  $\mu$ mol (1.1 mg) per mouse for four days – far in  
806 excess of what an equivalent human could realistically ingest <sup>152</sup>.

807 The juice extracts from Brassicaceae plants such as ‘salad’ rocket <sup>63</sup>, garden cress <sup>153</sup>  
808 and radish <sup>61</sup>, and their application to cancerous cell lines, such as colon cancer (HT-29) or  
809 hepatoma (HepG2) cells, are used to establish antigenotoxic, detoxification or  
810 antiproliferative effects. In rocket, it has been shown that extracts have protective effects  
811 against DNA damage in comet assays <sup>63</sup>. ITCs and their cysteine conjugates have shown  
812 efficacy in inhibiting HL-60 leukemia cells at concentrations as low as 0.8  $\mu$ mol.L<sup>-1</sup> <sup>150</sup>. In the  
813 use of other cell lines, the results are more mixed: some respond with an increase in CYP  
814 activity when exposed, whereas others do not <sup>149</sup>.

815 Similar effects have been associated with indolic-GSL breakdown products, such as  
816 I3C and 3,3'-diindolylmethane (DIM). Dietary studies conducted in rats have found that  
817 phase II detoxification enzymes are enhanced in the stomach, liver and small intestine after  
818 consumption of these compounds. Indoles are thought to act somewhat differently to ITCs  
819 however, inhibiting cancer cells through cytostatic mechanisms, rather than apoptosis <sup>96</sup>.

## **Consumer Health Benefits – Evidence From Human Clinical Trials & Epidemiology**

The increase in consumption of fruits and vegetables is accepted to be beneficial to human health <sup>154</sup>, but the compounds responsible and the interactions with genotype are not clear. It is assumed that what is beneficial for one person to consume, is beneficial for all people. This is not the case for many food types, and some evidence suggests it is the same for Brassicaceae vegetable consumption. It is known that human metabolic genotypes vary in the degree of beneficial effects that they will impart after ingestion of phytochemical compounds <sup>155</sup>, and adds an additional layer of complexity to producing Brassicaceae with enhanced GSL/ITC traits <sup>75</sup>.

The quantities required to elicit benefits in humans (both acute and chronic) are difficult to define due to variations in bioavailability within Brassicaceae food matrices and GSL-metabolism by gut microbiota in subjects <sup>156</sup>. The experimental quantities used in clinical research trials frequently do not translate into realistic or sustainable amounts that the average person can achieve. A study by Bogaards, Verhagen, & Willems <sup>157</sup> demonstrated that after human males consumed 300 g of Brussels sprouts per day, there was a significant increase in GST products in the blood compared to those on a GSL-free diet. While indicative of an underlying metabolic mechanism for ITC degradation, few people would be willing or able to consume such large Brussels sprout quantities on a daily basis. The impracticality of studies in the ‘real world’ and to ordinary people often detracts from the importance of the mechanistic findings. Doses are also often administered in a form that would not regularly be consumed (i.e. as a drink or powder supplement) <sup>158</sup>, which limits the relevance of results and the conclusions drawn. This raises the question: are the beneficial effects seen in trials ‘real-world’ effects, or just ones induced by extreme acute consumption?

Epidemiological studies looking at cancer risk vs. Brassicaceae vegetable consumption have reported mixed results. Studies in patients with prostate cancer, for

example, have found both significant inverse associations and no significant associations. For other cancers, such as endometrial, the risk reductions reported are moderate<sup>151</sup>. Data are encouraging, but do not identify or distinguish the modes of action that are responsible<sup>106</sup>. ITCs and indoles are strong candidates, but other compounds such as flavonoids, carotenoids and anthocyanins are also present in Brassicaceae. It is unlikely that these compounds act in isolation within the human body, and it may be the combined effect of ingesting a diverse range of phytochemicals contributes towards such risk reductions<sup>63</sup>.

Genetic studies on humans have identified several genes that play a role in ITC metabolism. GST loci and the associated *GSTM1*, *GSTT1* and *GSTP* genotype polymorphisms impact the relative protective effects of ITCs that an individual will receive. Individuals that are *GSTT1*-null and *GSTM1*-null are at higher risk of developing some cancers, such as renal cell carcinoma. Those who carry present copies of both *GSTT1* and *GSTM1*, and have only a low Brassicaceae intake, are still at a lower risk than null individuals by comparison<sup>151</sup>. It has been estimated that up to 40% of the population may benefit from increased Brassicaceae consumption due to the elevated risk associated with some null genotypes<sup>13</sup>. Breeding goals selecting for certain GSLs/ITCs have not considered consumer genotype as a variable, but in future this must be an expressed goal if populations are to gain full benefits of newly developed varieties<sup>75</sup>. This means that selection and enhancement for other compounds such as flavonoid glycosides, anthocyanins and carotenoids may be practical way of ensuring an ‘all-round’ health benefit to Brassicaceae crops.

It is well documented in clinical studies of raw vs. cooked vegetables that cancer risk (of multiple types) decreases with raw plant matter ingestion<sup>159</sup>. Consuming uncooked species (such as rocket or watercress) increases the contact between GSLs and myrosinase and the amounts of ITCs absorbed<sup>18</sup>. Due to the detrimental effects of cooking on GSLs and

myrosinase, *B. oleracea* crops may not be as effective/efficient as uncooked species at eliciting such reductions in overall risk.

The reported anticancer effects of Brassicaceae in the diet are poorly substantiated by empirical quantification of the total GSL/ITC amounts that are ingested and absorbed by the body, due to the potential variables previously outlined. A review of the health promoting properties of broccoli by Ares et al.<sup>160</sup> concluded that even with high broccoli intake, it is likely to be insufficient to stimulate anticancer effects at doses outlined in clinical studies. Broccoli varieties bred for high glucoraphanin content have showed promise however. It has been observed that doubling the level of glucoraphanin in florets can produce a three-fold increase in sulforaphane metabolites within the bloodstream compared with a standard variety<sup>155</sup>. This is supported by some excellent and rigorous human clinical studies with *Beneforté* broccoli, and have shown encouraging results<sup>161–163</sup>

## SUMMARY

Cell and animal studies have shown that ITCs and indoles have strong protective effects against some cancers<sup>164</sup>. Epidemiological evidence also suggests that vegetables containing GSLs are associated with reduced risks of developing cancer, heart disease<sup>165</sup> and neurodegenerative diseases<sup>10</sup>. These two kinds of studies are measuring very different things however. *In vitro* and *in vivo* animal studies often use ITC compounds in isolation and at high doses<sup>166</sup> measuring only acute effects. Epidemiological research often takes place over several years, and does not account for compounds acting in isolation (i.e. the beneficial effects cannot be wholly attributed to GSLs/ITCs)<sup>55</sup>. Flavonols, anthocyanins and carotenoids are but a few of the other classes present in these crops, and all have similar reported effects attributed to them<sup>96,167</sup>.

The health benefits a consumer receives from long-term Brassicaceae ingestion depends on the type and abundances of GSLs/ITCs/indoles within tissues. It depends on the environment in which these crops were grown, and their genetic predisposition for producing certain myrosinase breakdown products over others (i.e. ITC: nitrile ratio). It depends on how the crop is stored, prepared and cooked; it even depends on the metabolic genotype of the individual consumer. This therefore means that GSL measurement at harvest, as a proxy for ITCs/indoles at the time of consumption is extremely tenuous. It makes suggesting how much Brassicaceae should be consumed difficult and filled with caveats that are specific to the species in question and the person consuming it.

In order to breed new Brassicaceae varieties with enhanced health benefits, the concentrations and relative myrosinase hydrolysis product abundances must be considered<sup>75</sup>. The literature is plentiful in studies analysing and reporting GSL concentrations, but is lacking in corresponding ITC, nitrile and indole measurements. The predominant reason for this is that these compounds are difficult to extract, identify and quantify, due to their volatile/unstable nature and reactivity<sup>168</sup>. Simple methods have now been developed however, which give robust and informative results<sup>98,169</sup>. While the extraction methods take longer than a crude methanol GSL extraction, it is possible to analyse ITCs/nitriles easily by GC-MS. The information about these compounds will be vital to breeders in making informed selections for any possible health benefits. GSLs are a convenient proxy measurement for the types of breakdown products, but are not in-and-of themselves a good indicator of ITC:nitrile ratios, total abundances, or myrosinase activity.

In conclusion, the future of breeding for enhanced GSL/ITC Brassicaceae crops is positive due to the abundance of phenotypic variation available for selection by breeders, and the increased interest in developing health-beneficial products for the consumer. Consumers themselves are actively looking for such products, and are more aware about the long-term



effects of bad dietary habits<sup>170</sup>. As the development of *Beneforté* broccoli has demonstrated, breeding in this way is achievable for commercial Brassicaceae crops, but must be done in a holistic way which accounts for every stage of varietal development, commercial production, agronomic, and environmental factors – as well as the tastes, preferences and genotypes of the end consumer<sup>75</sup>. This may take decades to achieve, but a roadmap has been established.

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## Figure legends

**Figure 1.** A schematic of the most important factors for consideration when breeding for improved glucosinolate/isothiocyanate profiles of Brassicaceae species.

**Figure 2.** Molecular structures of isothiocyanates and indole compounds with known health-beneficial properties.

**Table 1.** Summary examples of glucosinolate content of edible crop species. Concentrations are expressed as mg.g<sup>-1</sup> dw of sinigrin. Values presented represent the average control concentration or raw material at the point of harvest unless otherwise stated. Values for leaves, sprouts, florets, stems and roots are presented separately.

Common name	Species	No. of cultivars tested	Environment	Glucoiberin	Progoitrin	Glucoraphenin	Glucoraphanin	Sinigrin	Glucoalyssin	Gluconapin	Diglucothiobeinin	Glucoiberverin	Glucosativin	4-hydroxyglucobrassicin	Glucolepidin	Glucobrassicinapin	Gluconapoleiferin	Glucotropaeolin	Dimeric glucosativin	Glucoerucin	Glucobrassicin	Gluconasturtiin	4-methoxyglucobrassicin	Neoglucobrassicin	Total	References	
Leaves																											
Ezo-wasabi	<i>Cardamine fauriei</i>	1	H	13.0						47.0											3.0				63.0	50	
Chinese cabbage	<i>Brassica rapa</i> var. <i>chinensis</i>	1	CE		nd		0.4	0.2	nd	0.7				nd		nd	nd			nd	<0.1	<0.1	nd	nd	1.9	36	
		23	F		0.6		nd	nd	nd	nd				nd		nd	nd			nd	0.8	1.7	nd	0.1	3.3	43	
		7	G		0.4		nd	<0.1	0.4	1.0				0.2		nd	nd			nd	0.5	0.1	0.8	<0.1	4.8	45	
	<i>Brassica rapa</i> var. <i>pekinensis</i>	23	G		0.4		nd	nd	nd	nd				nd		nd	nd			nd	0.5	2.1	nd	0.7	3.3	43	
		12	G		0.5		0.1	<0.1	0.5	nd				0.1		1.4	1.0			0.1	0.8	nd	1.3	0.1	5.9	44	
		1	?		nd		nd	nd	nd	nd				nd		nd	nd			nd	0.5	0.2	0.6	<0.1	1.4	31, 32	
	Average	-	-		0.3		0.1	<0.1	0.2	0.3				0.1		0.2	0.2			<0.1	0.5	0.7	0.5	0.2	3.4		
Salad rocket	<i>Eruca sativa</i>	28	CE		nd	<0.1	0.2		<0.1		<0.1	<0.1	3.9	<0.1	<0.1			<0.1	2.2	0.2					6.7	62	
		1	CE		nd	nd	0.3		nd		1.4	0.7	4.2	nd	nd			nd	nd	<0.1					6.6	63	
		1	CE		nd	nd	4.6		nd		nd	nd	10.8	nd	nd			nd	nd	2.9					18.3	64	
		21	G		0.8	nd	2.8		0.6		6.8	nd	3.3	nd	nd			nd	9.8	5.4					29.5	65	
		Average	-	-		0.2	tr	2.0		0.2		2.1	0.2	5.6	tr	tr			tr	3.0	2.1					15.3	

Table 1. Continued

Common name	Species	No. of cultivars tested	Environment	Glucobriferin	Progoitrin	Epi-progoitrin	Glucoraphenin	Glucoraphanin	Sinigrin	Glucosylsin	Gluconapin	Diglucothiobetin	Glucobrerverin	Glucosativin	4-hydroxyglucobrassicin	Glucolepidin	Glucotropaeolin	Dimeric glucosativin	Glucorucin	Glucobrassicin	Gluconasturtiin	4-methoxyglucobrassicin	Neoglucobrassicin	Total	References
Wild rocket	<i>Diplotaxis tenuifolia</i>	7	CE		nd		<0.1	0.2		nd		nd	<0.1	2.4	<0.1	<0.1	<0.1	4.7	0.2					7.7	62
		1	CE		nd		nd	0.4		nd			1.1	0.9	3.6	nd	nd	nd	nd	0.8				6.8	63
		16	G		0.4		nd	4.6		0.8			3.5	nd	2.0	nd	nd	nd	5.5	2.2				19.0	65
		Average	-	-		0.1		tr	1.7		0.3		1.5	0.3	2.7	tr	tr	tr	3.4	1.1				11.2	
Kale	<i>Brassica oleracea</i> var. <i>acephala</i>	1	CE	2.0	nd			0.4	0.4		nd				0.2				nd	2.1	<0.1	<0.1	0.2	5.3	52
		153	F	3.2	0.3			0.1	3.9		nd				nd				nd	2.9	<0.1	nd	0.3	10.7	37
		5	F	1.3	3.1			0.6	0.6		0.1				nd				<0.1	2.9	0.4	nd	nd	15.1	18
		2	G	1.1	<0.1			0.1	0.4		nd				0.1				nd	1.8	nd	0.1	0.3	3.9	19
		Average	-	-	1.9	0.9			0.3	1.3		<0.1				0.1				tr	2.4	0.1	<0.1	0.2	8.8
Cabbage	<i>Brassica oleracea</i> var. <i>capitata</i>	1	CE	nd	nd	nd		0.1	1.1	nd	0.1		nd		nd					0.1	<0.1	nd	nd	1.8	36
		26	F	2.7	0.3	0.3		<0.1	1.0	<0.1	<0.1		<0.1		<0.1					2.5	nd	nd	0.2	7.2	37
		6	F	0.2	0.6	nd		0.2	0.6	nd	0.2		<0.1		nd					0.8	nd	0.1	<0.1	2.5	1
		2	G	1.6	0.3	nd		1.1	1.7	nd	0.2		nd		0.1					2.6	nd	0.3	0.3	8.8	25
	<i>Brassica oleracea</i> var. <i>capitata</i> f. Savoy	1	?	2.9	0.1	nd		0.1	4.1	nd	nd		nd		nd					2.7	nd	0.5	nd	10.3	
		1	?	1.7	0.1	nd		0.1	1.7	0.2	0.2		nd		0.2					1.0	nd	0.7	<0.1	5.8	31, 32
		1	?	0.3	0.1	nd		0.1	<0.1	0.4	nd		nd		<0.1					0.4	nd	<0.1	nd	1.4	
		Average	-	-	1.3	0.2	<0.1		0.2	1.5	0.1	0.1		tr		<0.1					1.4	tr	0.2	0.1	4.1

Table 1. Continued

Common name	Species	No. of cultivars tested	Environment	Gluciberin	Progoitrin	Glucoraphanin	Sinigrin	Glucosylsin	Gluconapin	Gluciberverin	4-hydroxyglucobrassicin	Glucobrassicinapin	Glucorucin	Glucobrassicin	Gluconasturtiin	4-methoxyglucobrassicin	Neoglucobrassicin	Total	References
Red cabbage	<i>Brassica oleracea</i> var. <i>capitata</i> f. <i>rubra</i>	4	F	0.1	0.6	0.3	0.2	nd	0.3	<0.1	nd		nd	1.7	<0.1	0.1	0.2	3.4	1
		1	G/F	1.5	3.6	0.6	1.6	nd	1.4	nd	0.3		0.3	1.2	0.1	1.9	nd	18.4	39
		1	?	0.6	0.5	<0.1	1.1	0.1	0.2	nd	<0.1		nd	1.5	nd	0.1	nd	4.1	31, 32
		1	?	0.4	0.7	1.3	0.6	nd	1.3	nd	0.1		nd	0.2	nd	0.1	nd	4.7	40
		1*	?	nd	nd	1.1	1.3	nd	nd	nd	0.2		nd	0.3	nd	0.3	nd	3.0	41
		Average	-	-	0.5	1.1	0.7	1.0	<0.1	0.6	tr	0.1		0.1	1.0	<0.1	0.5	<0.1	6.7
White cabbage	<i>Brassica oleracea</i> var. <i>capitata</i> f. <i>alba</i>	?	?	1.2	0.3	nd	1.1	nd						nd		nd	nd	2.6	38
		1	?	2.7	0.1	0.1	1.7	<0.1						1.4		0.2	<0.1	6.1	31, 32
		Average	-	-	2.0	0.2	0.1	1.4	tr					0.7		0.1	tr	4.4	
Collards	<i>Brassica oleracea</i> var. <i>sabellica</i>	5	F	1.0	2.9	0.3	6.5		0.7					4.6	0.1			18.2	18, 19
Mustard greens	<i>Brassica juncea</i>	1	CE			nd	3.9		0.2					<0.1	0.1			4.3	36
		2	F			<0.1	29.3		0.2					0.3	0.3			47.4	18, 19
		Average	-	-			tr	16.6		0.2				0.2	0.2			25.9	
Leaf rape	<i>Brassica napus</i> var. <i>pabularia</i>	36	G		2.2			0.4	1.1			3.2		0.4				7.9	53

\* = Cultivars were purchased from multiple supermarkets but treated as one sample

Table 1. Continued

Common name	Species	No. of cultivars tested	Environment	Glucobriferin	Progoitrin	Glucoraphanin	Sinigrin	Glucosylsin	Glucosinabin	Gluconapin	Diglucothiobinin	Glucobriferin	Glucosativin	4-hydroxyglucobrassicin	Gluconapoleiferin	Dimeric glucosativin	Glucorucin	Glucobrassicin	Gluconasturtiin	4-methoxyglucobrassicin	Neoglucobrassicin	Glucomoringin	Acetyl glucomoringin (I, II, III)	3-hydroxypropyl GSL	Total	References	
Watercress	<i>Nasturtium officinale</i>	1	CE											nd				0.2	6.6	nd					7.1	36	
		1	H <sup>S</sup>												0.2				0.5	1.8	0.3				2.8	71	
		Average	-	-											0.1				0.4	4.2	0.2				5.0		
Chinese kale	<i>Brassica oleracea</i> var. <i>alboglabra</i>	1	F	0.1	1.9	4.0	0.1			7.6	<0.1			0.2	0.1		0.1	0.6		0.1	0.2			14.9	21		
Turnip rape	<i>Brassica napus</i>	1	CE				<0.1			4.8								0.1	0.7					5.6	36		
Dogmustard	<i>Erucastrum</i> spp.	1	G		<0.1	1.9		0.9			<0.1		<0.1			0.2	0.6							3.6	65		
Annual wall-rocket	<i>Diplotaxis muralis</i>	2	G		0.3	3.2		0.4			4.0		0.9			5.9	2.8							17.4			
White mustard	<i>Sinapis alba</i>	1	G				2.0		27.1															29.1	24		
Moringa	<i>Moringa oleifera</i>	6	F <sup>V</sup>																			50.2	9.3	59.5	60		
		30	F																			12.0	12.0	24.0	57		
		1	G/F																			17.1	11.8	28.9	59		
		6	G																			48.5	34.2	82.7	58 <sup>^</sup>		
		Average	-	-																			29.3	14.1	43.4		
Spider plant	<i>Cleome gynandra</i>	6	F																					3.1	3.1	69	
Ethiopian mustard	<i>Brassica carinata</i>	2	CE		<0.1		6.9	<0.1		<0.1				<0.1				0.1	nd	<0.1	<0.1			7.1	48		
		1	G		nd		1.3	nd		0.1				nd				0.2	<0.1	<0.1	<0.1			1.7	49		
		Average	-	-	tr		4.1	tr		0.1				tr				0.2	tr	<0.1	<0.1			4.4			
Sprouts																											
Broccoli	<i>Brassica oleracea</i> var. <i>italica</i>	1	CE	1.1	tr	18.3		<0.1		tr				4.0			3.9	5.5	tr	2.9	3.1			38.8	11		
		1	CE	2.9	nd	7.7		nd		nd				1.5			0.3	1.4	nd	3.5	1.6			18.9	15		
		Average	-	-	2.0	tr	13.0		tr		tr			2.75			2.1	3.5	tr	3.2	2.4			28.9			

\$ = cultivars were grown commercially in outdoor water beds; ^ = concentrations determined from reported % of total; V = grown in various geographical locations.



Table 1. Continued

Common name	Species	No. of cultivars tested	Environment	Gluciberin	Progoitrin	Epi-progoitrin	Glucoraphenin	Glucoraphanin	Sinigrin	Glucosylsin	Gluconapin	Glucoiberverin	4-hydroxyglucobrassicin	Glucobrassicinapin	Gluconapoleiferin	Glucorucin	Glucoraphasatin	Glucobrassicin	Gluconasturtiin	4-methoxyglucobrassicin	Neoglucobrassicin	Total	References
Turnip	<i>Brassica rapa</i> var. <i>rapa</i>	1	CE		4.2					0.1	0.8		2.4	tr	tr			2.3		2.2	2.4	15.0	11
Rutabaga	<i>Brassica napus</i> var. <i>rapifera</i>	1	CE		18.5						1.6		1.7	tr	tr			3.0		3.8	3.4	31.9	
China rose radish	<i>Raphanus sativus</i>	1	CE				3.3						1.5			0.2	41.1			2.7		48.8	
Radish		1	CE				16.7						2.7				17.2			tr		36.6	
Chinese kale	<i>Brassica oleracea</i> var. <i>alboglabra</i>	1	H	1.0	28.7			1.7	16.7		45.9		0.6		0.4	0.5		0.9		1.8	0.2	98.2	46
		2	?	1.2	11.9			nd	nd		15.9		nd		nd	nd		0.6		3.0	nd	32.8	47
		Average	-	-	1.1	20.3			0.9	8.4		30.9		0.3		0.2	0.3		0.8		2.4	0.1	65.5
Florets/Buds																							
Broccoli	<i>Brassica oleracea</i> var. <i>italica</i>	1	CE/F	1.7	nd	nd		17.4	nd	nd	nd	nd	1.8	nd	nd	nd		4.0	nd	0.8	1.2	26.9	16
		10	F	nd	0.4	nd		4.0	<0.1	nd	nd	nd	nd	nd	nd	nd		nd	nd	nd	nd	6.4	17
		6	F	0.3	0.1	nd		2.3	<0.1	nd	<0.1	nd	nd	nd	nd	0.1		2.3	<0.1	nd	nd	7.3	18, 19
		6	F	0.4	nd	nd		2.2	nd	nd	nd	nd	0.1	nd	nd	nd		1.4	nd	0.6	0.2	4.9	20
		4	F	0.2	0.3	nd		1.8	<0.1	nd	<0.1	nd	<0.1	nd	0.1	<0.1		0.9	nd	0.1	0.3	3.9	21
		1	F	0.3	nd	nd		1.7	nd	nd	nd	nd	0.1	nd	nd	nd		0.6	nd	0.1	0.3	3.1	22
		1	F	0.7	nd	nd		4.6	nd	nd	nd	nd	0.1	nd	nd	nd		1.7	nd	3.8	0.2	11.1	23

Table 1. Continued

Common name	Species	No. of cultivars tested	Environment	Glucoiberin	Progoitrin	Epi-progoitrin	Glucoraphanin	Sinigrin	Glucosylsin	Gluconapin	Glucoiberverin	4-hydroxyglucobrassicin	Glucoibassicinapin	Gluconapoleiferin	Glucotropaeolin	Glucocerucin	Glucobrassicin	Gluconasturtiin	4-methoxyglucobrassicin	Neoglucobrassicin	Total	References
Broccoli (continued)	<i>Brassica oleracea</i> var. <i>italica</i>	1	F	nd	nd	nd	0.9	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	<0.1	nd	nd	1.0	24
		2	G	nd	nd	nd	4.6	nd	nd	nd	nd	0.2	nd	nd	nd	nd	2.6	nd	0.3	1.9	9.3	25
		148	G	0.1	0.9	0.6	1.4	<0.1	<0.1	0.1	nd	0.1	nd	nd	nd	0.1	2.0	nd	0.3	0.7	5.8	87
		50	G/F	<0.1	0.1	nd	2.8	<0.1	0.1	0.4	nd	0.1	0.1	0.3	nd	nd	0.4	0.2	0.2	0.1	5.1	26, 27
		-	M	1.6	3.2	<0.1	7.7	<0.1	1.2	0.2	<0.1	0.1	<0.1	0.2	nd	<0.1	6.9	0.1	0.4	4.0	25.6	28 <sup>+</sup>
		2	?	nd	nd	nd	8.3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	8.3	29
		-	?	nd	nd	nd	1.9	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.4	30
		1	?	0.2	2.3	nd	0.1	nd	2.3	0.2	nd	nd	nd	nd	nd	nd	0.8	nd	0.1	0.4	6.6	31, 32
		1	?	1.3	nd	nd	3.2	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.4	nd	0.1	0.6	5.6	33
		1	?	0.2	nd	nd	0.9	nd	nd	nd	nd	0.1	nd	nd	nd	nd	0.7	nd	0.4	0.2	2.5	34
		1	?	0.3	nd	nd	2.8	nd	nd	nd	nd	0.1	nd	nd	nd	nd	2.3	nd	0.3	0.9	6.9	14
Average		-	-	0.4	0.4	<0.1	3.8	0.4	0.2	<0.1	tr	0.1	<0.1	<0.1	nd	<0.1	1.5	<0.1	0.4	0.5	7.9	
Brussels sprouts	<i>Brassica oleracea</i> var. <i>gemmifera</i>	6	F	0.4	0.5	nd	0.5	0.5	nd	0.3	<0.1	nd	nd	nd	nd	<0.1	15.8	<0.1	nd	nd	22.4	18, 19
		1	F	0.1	2.1	nd	10.5	nd	0.9	nd	nd	nd	nd	nd	0.4	nd	2.6	0.2	0.7	0.1	17.6	35
		2	G	1.4	0.8	nd	0.6	0.9	nd	0.3	nd	0.2	nd	nd	nd	nd	4.4	nd	0.4	0.2	10.3	25
		1	?	1.2	3.0	nd	0.1	3.3	0.8	2.8	nd	0.5	nd	nd	nd	nd	1.8	0.1	0.6	nd	13.9	31, 32
		1	?	0.9	nd	nd	0.2	nd	nd	nd	nd	0.1	nd	nd	nd	nd	0.9	nd	nd	0.1	2.2	34
		Average		-	-	0.8	1.3	nd	0.3	3.0	0.2	0.9	tr	0.2	nd	nd	0.1	tr	5.1	0.1	0.3	0.1

+ = Median values taken from range data

Table 1. Continued

Common name	Species	No. of cultivars tested	Environment	Glucobriferin	Progoitrin	Glucoraphanin	Sinigrin	Glucobacteroin	Glucosylsin	Glucosinalbin	Gluconapin	Glucobriferin	4-hydroxyglucobrassicin	Glucobrassicinapin	Gluconapoleiferin	Glucotropacolin	Glucolimnathin	Glucoerucin	Glucoraphasatin	Glucobrassicin	Gluconasturtiin	4-methoxyglucobrassicin	Neoglucobrassicin	Glucomororingin	Acetyl glucomororingin (I, II, III)	3-hydroxypropyl GSL	Total	References			
Cauliflower	<i>Brassica oleracea</i> var. <i>botrytis</i>	5	F	0.5	nd	<0.1	0.3				<0.1	0.2	nd		nd			<0.1	2.5	<0.1	nd	nd					4.0	18, 19			
		4	F	0.9	0.1	0.4	0.1				<0.1	0.1	<0.1		<0.1			<0.1	0.9	nd	0.1	0.1					2.9	21			
		1	F	0.7	0.1	0.1	0.7				nd	nd	nd		nd			nd	0.9	nd	<0.1	<0.1					2.5	22			
		2	G	0.6	0.1	<0.1	0.8				<0.1	nd	0.2		nd			nd	2.3	nd	0.2	1.5					5.8	25			
		5	G/F	1.3	2.5	0.9	0.6				nd	nd	0.7		nd			0.1	4.1	nd	0.7	0.5					11.4	42			
		1	?	0.1	nd	0.1	0.1				nd	nd	<0.1		nd			nd	0.3	nd	0.1	<0.1					0.7	31, 32			
		1	?	0.4	nd	<0.1	nd				nd	nd	<0.1		nd			nd	0.7	nd	0.2	0.1					1.5	34			
Average	-	-	0.6	0.4	0.2	0.4				tr	<0.1	0.1		tr			<0.1	1.7	tr	0.2	0.3					4.1					
Stem																															
Kohlrabi	<i>Brassica oleracea</i> var. <i>gongylodes</i>	1	F	0.1	<0.1	0.2		nd				0.4	nd					1.3	1.1	0.1	nd	nd					3.4	18, 19			
		1	?	0.1	nd	<0.1		0.1				nd	<0.1					nd	0.5	nd	<0.1	0.2					1.0	31, 32			
		Average	-	-	0.1	tr	0.1		0.1			0.2	tr					0.7	0.8	0.1	tr	0.1					2.2				
Moringa	<i>Moringa oleifera</i>	1	F																								16.3	4.8	21.1	60	
Spider plant	<i>Cleome gynandra</i>	8	F																								7.6	7.6	69		
Ethiopian mustard	<i>Brassica carinata</i>	1	G				2.8				0.4		0.1						0.2	2.3	0.1	0.1					6.0	49			
Root																															
Rutabaga	<i>Brassica oleracea</i> var. <i>rapifera</i>	1	CE		2.8	nd		1.2	0.2					0.1	0.1	0.4		0.4		0.2	nd	0.1	0.1					5.6	68		
		1	?		0.9	0.3		nd	nd					0.1	nd	nd		nd		0.4	1.2	0.1	0.4					3.5	31, 32		
		Average	-	-		1.9	0.2		0.6	0.1				0.1	0.1	0.2		0.2		0.3	0.6	0.1	0.3					4.6			
Maca	<i>Lepidium meyenii</i>	3	F					0.1	0.2							6.9	1.5										8.6	54			
Radish	<i>Raphanus sativus</i>	1	?	0.1		0.1													1.9	0.1	0.1	0.2					2.8	31, 32			
Moringa	<i>Moringa oleifera</i>	3	F <sup>V</sup>																						8.6	10.2	18.8	60			

**Table 2.** Summary of factors that influence glucosinolate composition of Brassicaceae plants during cultivation

Variable	Species		
	Broccoli	Reference	Cauliflower
<b>Genotype</b>	↑ Indole GSLs ↑ Alkyl GSLs	86	↑Indole GSLs
	Significant differences in total GSLs, indole GSLs & glucoraphanin	25	Significant differences in total GSLs, indole GSLs & glucoraphanin
	Significant differences among cultivars for alkyl, alkenyl, indole and total GSLs.	21	Significant differences among cultivars for alkyl, alkenyl, indole and total GSLs.
	Significant differences between individual GSL concentrations between cultivars	87	
<b>Environmental temperature</b>	↑ Total GSLs at low temp. ~14°C	86	↑Total GSLs at low temp. ~14°C
	↓Total GSLs with increasing temperature	25	↓Total GSLs with increasing temperature
	Variability of individual GSLs according to temp.	16	
<b>Light intensity</b>	↑Total GSLs at high light levels (450 $\mu\text{mol m}^{-2}\text{s}^{-1}$ )	86	↑Total GSLs at high light levels (450 $\mu\text{mol m}^{-2}\text{s}^{-1}$ )
	Total & indole GSLs influenced by day length & light intensity	25	
	↓Glucoraphanin with high light at harvest		
	↑Total GSLs with light	115	Total & indole GSLs influenced by day length & light intensity
	Variability of individual GSLs according to day length	16	↓Glucoraphanin with high light at harvest

↑Increase; ↔no-effect; ↓decrease

Table 2. Continued

Variable	Species					
	Broccoli	Reference	Cauliflower	Reference	Radish	Reference
Sulfur application	↑Alkyl & indole GSLs (600 mg S per plant)	86				
	↔ (150 kg S ha <sup>-1</sup> )					
	↔ Low S (15 kg S ha <sup>-1</sup> )	115	-	-	↑Alkenyl GSLs (30 mg S per plant)	86
	↑Aliphatic & total GSLs (>15 mg.L <sup>-1</sup> )	15				
Nitrogen application	↑Total GSLs with reduced N	86				
	↑Total GSLs with reduced N (1g N per plant)	115	↑Total GSLs with reduced N	86	↑Total GSLs with reduced N	86
Selenium application	↑Total GSLs (5.2 mM Se)	115				
	↔	127	-	-	↑Total GSLs & glucoraphanin in soil ↓Total GSLs in hydroponics	129
Water availability	↑Total GSLs with reduced water	86				
	↑Total GSLs with severe drought	115	↑Total GSLs with reduced water	86	↑Total GSLs with reduced water	86
Soil salinity	↑Total GSLs (40, 80mM)	115	-	-	-	-
Season	↑Total GSLs in spring & autumn	86	↑Total GSLs in spring & autumn	86	↔	86
Amino acid supplementation	↑Alkyl GSLs with methionine	86	-	-	↑Alkenyl GSLs with methionine	86
Developmental stage	↑Indole GSLs in immature florets	85				
	↑Glucoraphanin between transplanting & harvest	25	↑Glucoraphanin between transplanting & harvest	25	↔	86

↑Increase; ↔no-effect; ↓decrease

Table 2. Continued

Variable	Species					
	Cabbage	Reference	Brussels sprouts	Reference	Wild rocket	Reference
Genotype	↑Sinigrin content in some varieties	37	Significant differences in total GSLs, indole GSLs & glucoraphanin	25	Significant differences between genotypes for aliphatic and total GSLs	62
	Significant differences in total GSLs, indole GSLs & glucoraphanin	25				
Environmental temperature	↓Total GSLs with increasing temperature	25	↓Total GSLs with increasing temperature	25	-	-
	↑Total GSLs at 32°C	115				
Light intensity	Total & indole GSLs influenced by day length & light intensity	25	Total & indole GSLs influenced by day length & light intensity ↓Glucoraphanin with high light at harvest	25	↔	63
	↓Glucoraphanin with high light at harvest					
	↑Total GSLs during the night	115				
	↓Total GSLs during the day					
Selenium application	↔	127	-	-	-	-
Water availability	↑Total GSLs with severe drought	115	↔No effect under mild drought	25	-	-
	↓Total GSLs under mild and severe drought					
Season	↑Glucoiberin & glucobrassicin in spring	37	-	-	-	-
	↑Total GSL in spring					
	↑Total GSL in spring	1				
	↑Indolic GSLs in fall					
Developmental stage	↑Glucoraphanin between transplanting & harvest	25	↑Glucoraphanin between transplanting & harvest	25	-	-

↑Increase; ↔no-effect; ↓decrease

Table 2. Continued

Species						
Variable	Ezo-wasabi	Reference	Salad rocket	Reference	Kale	Reference
Genotype	-	-	Significant differences between genotypes for aliphatic and total GSLs	62	Significant differences in total GSLs, indole GSLs & glucoraphanin	25
Environmental temperature	-	-	-	-	↓Total GSLs with increasing temperature ↓Total GSLs at cold temperatures (9-15°C)	25 52
Light intensity	↑Total GSLs; red+blue light ↑Indolic:aliphatic GSL ratio; red or green light ↑Aliphatic, ↓indolic GSLs; blue light	50	↔	63	Total & indole GSLs influenced by day length & light intensity ↓Glucoraphanin with high light at harvest	25
Developmental stage	-	-	-	-	↑Glucoraphanin between transplanting & harvest	25
Variable	Turnip	Reference	Ethiopian mustard	Reference	Thale cress	Reference
Light intensity	-	-	-	-	↑Total GSLs with light ↓Total GSLs in the dark	115
Sulfur application	↑Total GSLs (60 kg S ha <sup>-1</sup> )	115	-	-	-	-
Potassium application	↓Total GSLs with K deficiency	-	-	-	↑Total GSLs with K deficiency	115
Water availability	↑Total GSLs with mild drought	115	↔ No effect under mild drought ↑Total GSLs with severe drought	86, 115	↓Total GSLs under mild and severe drought	115

↑Increase; ↔no-effect; ↓decrease

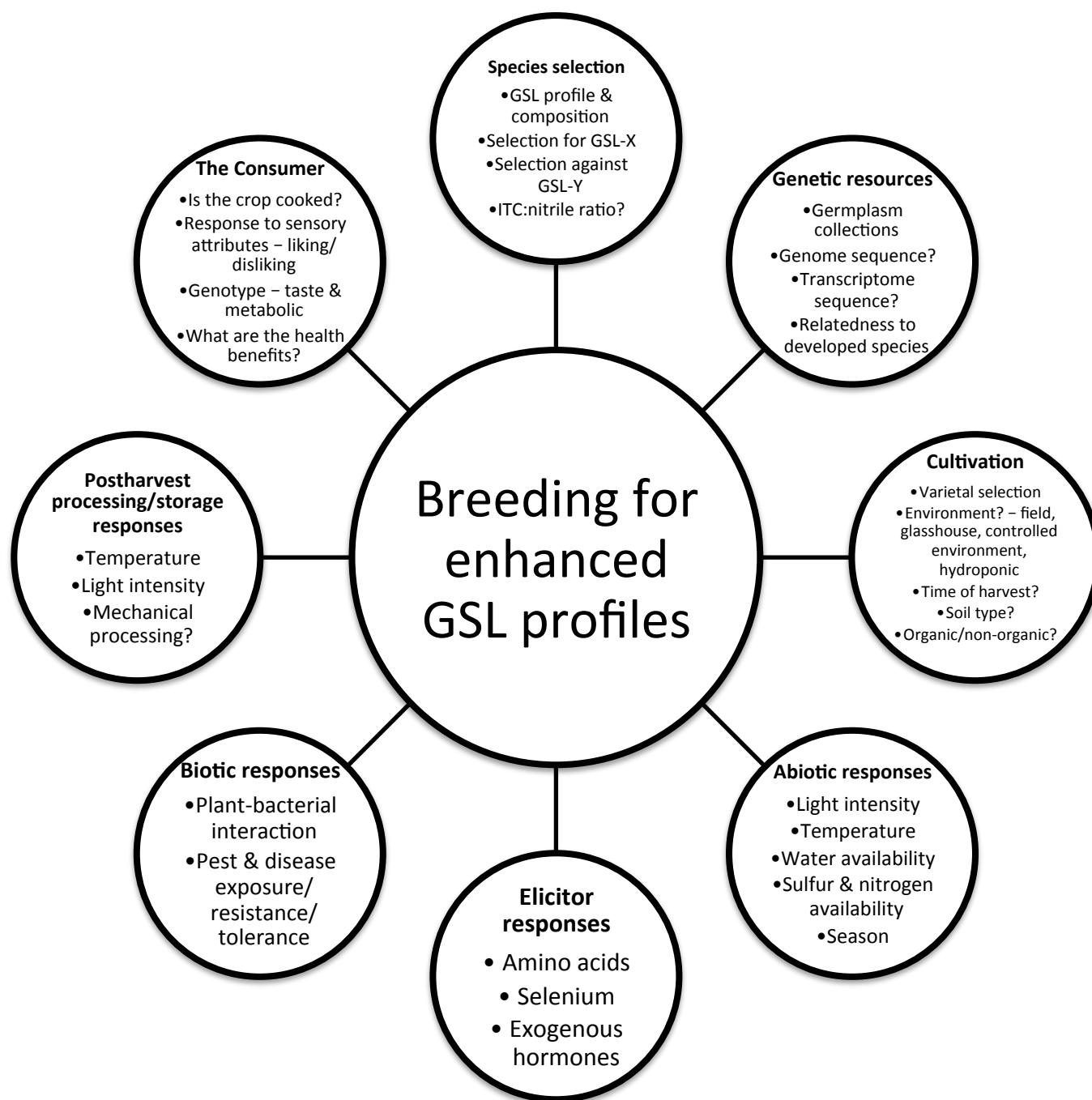
Table 2. Continued.

Species						
Variable	Swede	Reference	Chinese cabbage	Reference	Rapeseed	Reference
Genotype	-	-	Significant differences between genotypes for glucobrassicin and gluconasturtiin	43	-	-
			Total and indolic glucosinolates vary between genotypes	44		
Environmental temperature	↑Progoitrin & glucobrassicin at 21°C	68	↑Total GSLs between 21-34°C ↓Total GSLs between 15-27°C	115	-	-
Water availability	-	-	-	-	↑Total GSLs with severe drought ↔ No effect under mild drought	115
Soil salinity	-	-	↑Total GSLs (40, 80mM)	115	-	-
Variable	White mustard	Reference	Chinese kale	Reference		
Genotype	-	-	Significant differences among cultivars for alkyl, alkenyl, indole and total GSLs.	21		
Light intensity	-	-	↓Gluconapin under blue light ↑Glucoraphanin under blue light	46		
Selenium application	↔	127	-	-		

↑Increase; ↔no-effect; ↓decrease

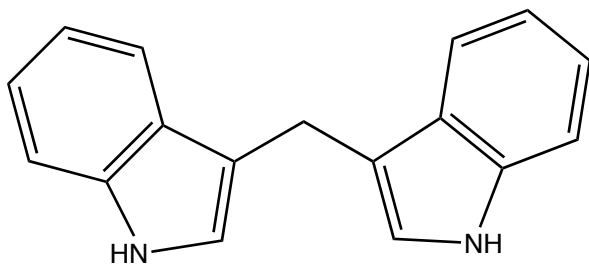


**Figure 1**

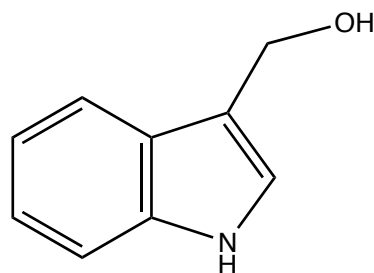


**Figure 2.**

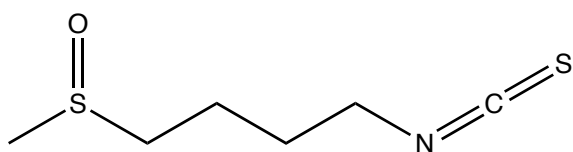
**3,3'-diindolylmethane (DIM)**



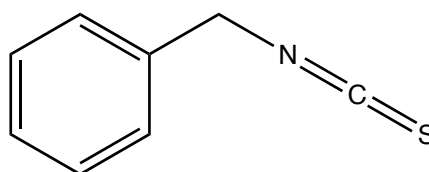
**Indole-3-carbinol (I3C)**



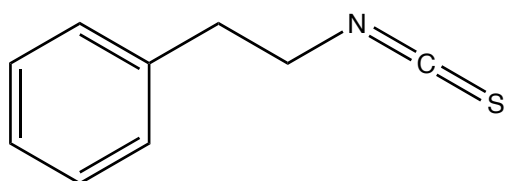
**Sulforaphane (SFN)**



**Benzyl isothiocyanate (BITC)**



**Phenethyl isothiocyanate (PEITC)**



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